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COMPARATIVE RESPONSES OF LONG-DAY AND SHORT-DAY PLANTS TO RELATIVE LENGTH OF DAY AND NIGHT[1]

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The primary purpose of the present discussion is to contrast the behavior of the so-called long-day and short-day types of plants in terms of their response to differences in length of day and to consider the problem of satisfactorily classifying plants into these two groups. In the first paper by GARNER and ALLARD dealing with the effect of length of day on plant growth, published about 12 years ago, two broad facts were brought out with respect to initiation of sexual reproduction, namely: (1) that some plants are more sensitive than others to the length-of-day factor; (2) that of the more sensitive group some respond to relatively long days while others respond to relatively short days. Thus we have one group embracing what may be designated as the indeterminate type of plant and a second group which can be conveniently divided into the long-day type and the short-day type. This classification has proved helpful and it seems desirable that it be retained, at least until some better system is devised. It appears, however, that unless a better understanding can be reached as to the basis on which the classification rests, considerable confusion is likely to result.

In dealing with the action of length of day on plant growth, greatest interest naturally attaches to its formative effects, and in the writer's own studies the action of the light period in initiating or suppressing sexual reproduction has been stressed.) It is on these effects that our classification of plants into long-day and short-day groups is based. In a recent general review of photoperiodism in plants, SCHICK[2] suggests that for those plants in which tuber formation is of importance, this reaction to the light period rather than flower formation be used as a basis for classification, and this

[1] Second STEPHEN HALES address; read before the American Society of Plant Physiologists at Atlantic City, December 30, 1932.

[2] SCHICK, R. Photoperiodismus. Der Züchter 4: 122–135. 1932.

proposal seems logical. On the other hand, some recent investigators have grouped their experimental material into long-day and short-day types on the basis of the growth rate under different day lengths. Obviously this represents an entirely different system of classification. Again, some investigators in this field have shown an inclination to apply the designation "long-day plants" to experimental material which has been merely exposed to a long-day treatment without regard to the response obtained, and, likewise, plants exposed experimentally to a short day would be designated as "short-day plants."

Assuming that initiation of processes of reproduction is accepted as the basis for classification, it might seem at first thought that long-day plants could be defined simply as those which respond to long days (or even continuous illumination) and short-day plants as those responding to short days. We should then be confronted, however, with the problem of determining what constitutes a long day or a short day. For example, the equatorial or equinoctial day length of 12 hours cannot be regarded as furnishing a satisfactory dividing line, for this would throw into the long-day group a very large proportion of the plants which on the basis of their photoperiodic responses could be regarded as properly belonging only in the short-day group. Again, if the line of separation between short days and long days be moved up, say to a light period of 13 or 14 hours, the lower limits for flowering in many plants regarded as really belonging in the long-day group would fall below this period. The fact is that there is a range in day length from about 12 to 14 hours which is common ground for many plants in both groups, in that they are able to flower within this range even though in most cases it would not be optimal for either group. Because of its extreme simplicity (at least in principle), it may be that this plan of arbitrarily designating a particular day length as a basis for separating the long-day and short-day types will persist despite its obvious weaknesses. If so, perhaps a 14-hour day may well be adopted to supply the dividing line.

Critical length of day for flowering

It is believed that the problem of satisfactorily separating the two groups of plants may be more logically approached from a somewhat different angle. While it seems to have been the impression of some that in general the more sensitive plants require a particular daylight period for successful flowering, ordinarily this is not the case. It is true that there is a fairly definite optimum length of day for flowering, but generally speaking there is also a rather wide range in day length on either side of the optimum in which flowering takes place with more or less facility. Through these ranges, differences in time required for attainment of the flowering stage,

though appreciable, are not large. To take a concrete case, the Biloxi variety of soy beans, a late-flowering or short-day type, has been found to have an optimum day length for flowering of about 9 hours; but under favorable conditions it is capable of flowering under all lengths of day, ranging from 5 to 12 hours, with a maximum delay of only about 7 or 10 days as compared with results under the optimum length of day. While there may be a few exceptions, the great majority of plants of both the long-day and the short-day type show these characteristics. In contrast with these relations, however, there exist in the case of the more sensitive plants what may be called critical day lengths for flowering which have a much narrower range for any particular plant. Taking again the Biloxi soy bean, the critical daylight period may be considered as lying between 13 and 14 hours. Under a 13-hour daily light period the plant flowers after a delay of some 10 or 12 days as compared with optimum conditions of day length. With a daylight period of 14 hours the critical point has been passed and the plant tends to become strictly vegetative, flowering being delayed for a more or less indefinite time. Likewise garden balsam flowers readily with a day length of about 14 hours but is greatly delayed in flowering with a day length of 13½ hours, and with shorter day lengths it becomes strictly vegetative. In many species representing both the long-day and the short-day types, it has been found that under suitable conditions a variation in day length of not more than one hour (or even less) constitutes the critical range, on the two sides of which definite contrast in response is obtained. On the one side the plant flowers readily while on the other side it tends to remain in the vegetative stage. The important point in this connection is that the group of plants which we have been in the habit of classing as the long-day type flower only when exposed to day lengths in excess of the critical, while the short-day plants are able to flower only under shorter day lengths than the critical. In the present stage of our knowledge of the subject this would seem to furnish a simple and logical basis for differentiating between the two groups of plants. It is to be noted that this method of classification is independent of any particular relationship between the actual duration of the critical light periods for the two types of plants, and it is not necessary to determine what constitutes a long day or a short day. From this point of view the decisive factor is not the actual length of the critical light period but the type of the response to light periods on the two sides of the critical. Thus it is even possible for the critical light period of a long-day type to be shorter than that of a short-day plant, although in the more typical cases this will not hold true. In general, designation of the two groups as long-day and short-day types is still justified under this method of classification.

Classification into long-day and short-day types and the factor of latitude

In applying the method of classification here proposed, it is necessary only to compare the character of the responses to a light period in excess of the critical and to one shorter than the critical. This is not necessarily the same, however, as merely determining the responses to what would be considered relatively long and short day lengths at a given latitude. Of course, we do not know the extreme range in length of day needed to embrace the critical light periods of all plants, and there is less information as to the upper limits than as to the lower ranges required. Nevertheless the indications are that few plants have a critical daylight period of much less than 12 hours, and it seems safe to assume that a 10-hour day would provide an exposure well below the critical for nearly all plants. As to upper limits, it has been found that some plants have a critical daylight period as long as 17 hours, so that evidently a day length of at least 18 hours must be used to provide an exposure distinctly in excess of the critical. If it were not for the disturbing factor of change in maximum length of day with change of latitude, for purposes of orientation in a given locality it would be necessary only to compare the behavior of the test plant under a 10-hour day and the full summer-day length. If flowering be materially delayed under the short-day treatment, the plant is to be regarded as belonging to the long-day type, while it would be placed in the short-day group should the short-day treatment hasten flowering. In the event that the date of flowering remain substantially the same under the two treatments, the plant would have to be regarded as belonging to what may be termed the indeterminate group. But a test of this sort will ordinarily give a reasonably dependable conclusion only when marked differences in response under the two treatments are obtained.

In other words, if the test definitely indicates that the plant in question belongs either to the long-day or the short-day group, the result may be considered fairly conclusive. Unless the experiment is conducted at very high latitudes, however, a result indicating that the plant is not sensitive to the day-length factor is to be accepted as purely tentative. This is true because there can be no assurance that the maximum summer-day length exceeds the critical light period. In working in lower latitudes, however, this difficulty can easily be overcome by employing artificial light to prolong the illumination period to, say, 18 hours. This will certainly take care of the great majority of cases although it is possible that in rare instances additional intermediate light exposures will be required.

Indeterminate group of plants

This group may be regarded as embracing those plants which are capable of flowering more or less readily throughout the range in length

of day ordinarily encountered through the year and at different latitudes. In such plants the day-length effects are primarily of a quantitative rather than a qualitative nature. The size attained by the plant and the extent or amount of reproductive activities and other growth features may be materially influenced, so that such plants may have an optimum day length for flowering. The essential point is that this group has no critical light period with respect to flowering, and under suitable growing conditions its members tend to flower at practically all latitudes and at all seasons of the year. The plants of this group are designated as "indeterminate" for the reason that the length-of-day factor does not exercise a clearly defined determinative (formative) effect, while they also might be designated as intermediate in the sense that in some respects the plants resemble both the long-day and short-day types and serve as a sort of connecting link between the two.

In attempting to utilize the critical light-period relations as a basis for grouping plants with respect to the day-length response, the mistake is likely to be made of throwing into the indeterminate group many plants which do not properly belong there unless the precautions previously mentioned are applied. This is what will usually occur when all light exposures employed for purposes of orientation happen to fall on one side of the critical light period. It will be recalled that in general short-day plants are able to flower with more or less facility through a wide range in day lengths below the critical, while the same is true of long-day plants for day lengths above the critical. In each case the response within these ranges is such as to indicate that there is no critical light period and the plants simulate the behavior of the indeterminate group. These relationships are well illustrated by the responses to differences in day length of the early, medium, and late varieties of soy beans, the Mandarin, Peking, and Biloxi varieties respectively. In plantings made in the greenhouse at frequent intervals throughout the year at Washington, the Mandarin has not been materially affected as to time required for flowering by the seasonal change in length of day, which ranges from 9½ to 15 hours. This variety therefore shows the characteristics of the indeterminate group when grown at Washington. It has been shown, however, that the Mandarin has a definite critical light period of about 17 hours and at greater day lengths flowering ceases. Hence, at a latitude of about 52°, as in southern Canada, this variety together with the Peking and Biloxi varieties may be expected to show the characteristics of a typical short-day plant. In Washington, at latitude 39°, the Peking and Biloxi continue to behave as short-day plants. In northern Florida, with a maximum summer-day length of about 14 hours, the Peking presumably would cease to respond as a short-day plant and would simulate the behavior of the indeterminate group. Finally, the

Biloxi, because of its relatively short critical light period, apparently would continue to act as a short-day type considerably farther southward. The Mandarin soy bean illustrates the fact that according to the proposed system of classification a short-day plant may have a relatively very long critical light period.

It remains to point out that undoubtedly some plants are injured by a very long daily light period or by continuous illumination; and possibly there are some which are so sensitive to the factor of day length that the range in light periods favorable to flowering is rather narrow, so that day lengths both too long and too short would occur. If such plants exist they must have two critical light periods, one on either side of the optimum period for flowering. These plants obviously would have the characteristics of both the short-day and the long-day types under this method of classification, and it might become necessary to place them in a special group.

Types of response in short-day and long-day plants to day lengths unfavorable for flowering

It is characteristic of a large group of herbaceous plants that the preflowering period of vegetative activity is limited chiefly to a leaf-rosette stage, and normally elongation of the axis is more or less rapidly followed by flowering. In another group stem elongation is a prominent feature of the early stages of growth, and under some conditions the axis may continue to elongate for a prolonged period without the appearance of flowering. This last-named group seems to furnish typical short-day plants while the first group supplies typical long-day plants. Thus, when exposed to a day length in excess of the critical, there is in the short-day type pronounced, long-continued elongation of the stem without flowering, while exposure to a day length shorter than the critical quickly initiates reproductive activity. On the other hand, in the long-day plant, exposure to a length of day in excess of the critical results in elongation of the axis, which is promptly followed by flowering; while exposure to a length of day below the critical tends to limit development to a leaf-rosette stage. It would seem, therefore, that the plan of classification herein presented is more or less in accordance with lines of division seen in nature.

It is apparent that when grown at different latitudes or at different seasons of the year, plants of both the long-day and the short-day types as measured by their responses may frequently shift from their true positions into the indeterminate group. No instance has been found, however, in which changes in the daylight period are capable of causing a long-day plant to behave as a short-day type or a short-day plant to simulate the behavior of a long-day type. As already pointed out, it is possible that some highly sensitive plants will be found to combine the characteristics of both

types. Such plants typically would exhibit the leaf-rosette form under a length of day shorter than the lower critical light period; a day length between the two critical periods would produce a flowering stem; while day lengths in excess of the upper critical period would lead to extensive elongation of the axis without flowering.

Interrelation of length of day and temperature

Normal response to the day-length factor of course will be dependent upon favorable conditions of temperature. While other factors of the environment may be operative in varying degrees, in nature the combined action of length of day and temperature appears to be of special importance. For present purposes it will be sufficient to consider this factor complex very briefly in its relation to adaptation of the long-day and short-day types to different latitudes and different seasons of the year. The length-of-day factor is essentially constant from year to year while the seasonal temperature usually is variable. Hence under ordinary circumstances temperature may be regarded as more commonly responsible for variations in plant growth and development from year to year, particularly with respect to such features as time of flowering. Nevertheless, through the seasons of the year there is usually a definite trend in temperature, and it may be noted that ordinarily the mean-temperature curve for a period of years in its course from month to month rather closely resembles the length-of-day curve. There is, however, one significant point of difference. There is a pronounced lag in temperature change as compared with change in length of day which, at Washington for example, amounts to some 3 or 4 weeks.

Beginning with the late winter season, the rapidly increasing length of day in regions beyond the tropics will tend to stimulate plant life to renewed growth, while in the main the lag in temperature rise will operate to retard growth. Initially the day length is short and hence favors flowering in the short-day type of plant but is unfavorable for flowering in the long-day type. With rapid increase in the daylight period with advance of season, successful flowering in the short-day type becomes essentially a question of whether the favorable range in day length will have passed before the temperature has arisen sufficiently to permit definite expression. Naturally this may depend at times upon whether the spring season happens to be relatively warm or cold. In the main it may be said that at high latitudes conditions are not favorable for successful spring flowering in short-day plants, although those which are capable of growing at relatively low temperatures have a distinct advantage over the less hardy forms. The situation is different with respect to long-day plants, for the increasing length of day with advance of the season is constantly becoming more favorable for flowering. The long days of late spring and early

summer of course are favorable for flowering only in the long-day group of plants. As the days begin to shorten in late summer, the conditions become particularly favorable for flowering in the short-day group. Here the distinct lag in fall of temperature as compared with decrease in day length is especially advantageous to this group. Successful flowering and fruiting will depend chiefly upon whether the plant is capable of responding to the day-length factor early enough for completion of the reproductive processes before the advent of cold weather. Of course, chances of successful reproduction are greater in those species which are capable of tolerating cool temperatures. Summarizing, it may be stated that with increasing latitude the conditions become less favorable for flowering in short-day plants, and particularly so in the spring because of the combined effect of day length and temperature. For those of the short-day group having a moderately high critical light period, more favorable conditions for flowering will occur in late summer or early fall. Long-day plants having a very high critical light period cannot well flower and fruit successfully except at high latitudes, and capacity to grow under cool temperatures is distinctly advantageous. On the whole, of course, the short-day group is best adapted to relatively low latitudes and the long-day group will be more successful in higher latitudes. In this connection the work of LUBIMENKO, MAXIMOW, and associates in Russia, TINCKER and associates in England, MCCLELLAND in Puerto Rico, YOSHII, KONDO, and associates in Japan, SCHICK in Germany, and others seems to lend support to this theory that the length-of-day factor plays an important rôle in the natural distribution of plant species and in crop-plant adaptation.

Effects of abnormal light periods

One of the most interesting observations on the length-of-day effect, which was made in the early stages of these investigations, is that when reduction in the number of hours of light received by the plant is accomplished by midday darkening, the striking effects on flowering obtained by excluding the early morning or late afternoon light are no longer seen. It has been demonstrated that this result involves primarily the light period and not changes in intensity or spectral composition of the illumination. The midday darkening does not initiate flowering in the short-day type, and usually it does not materially interfere with reproductive processes in the long-day type, even when the period of darkening is long enough to impair the general nutrition of the plant. Evidently the net result of this method of reducing the daily number of hours of illumination is to favor vegetative activity in short-day plants and to favor reproductive activity in long-day plants. In a sense the effects of the usual short-day and long-day treatments are reversed. Although the number of hours of daily illumination

totals only that constituting a day of 9 or 10 hours, the effects on both groups of plants are those of a long day.

In this treatment the plants are exposed to the normal night period of darkness and to a midday period of darkness, with short periods of illumination in the forenoon and afternoon, so that the light relations are rather complex. The same results, however, have been obtained consistently when long-day and short-day plants were exposed to short, equal alternations of light and darkness obtained by use of electric light. With natural illumination the condition of equal day and night is not far removed from the optimum for initiation of flowering in most plants of the short-day type, but this 12-hour day is more or less unfavorable for flowering in the long-day group. Similar relations have been found to apply when artificial illumination is used. On the other hand, when the equal alternations of light and darkness were reduced to 6 hours or 4 hours, flowering was hastened in long-day plants and delayed in short-day plants. As the periods of light and darkness were further shortened these results were accentuated. Thus all short alternations of light and darkness, extending down to those as short as 5 seconds, proved to be unfavorable for flowering in the short-day group but distinctly favorable in the long-day type. These relations persisted even though in some of the treatments the nutrition of the plant was seriously disturbed. It is evident that here, as in the case of midday darkening, exposure to short alternations of light and darkness produces results in both groups of plants similar to those obtained with a long day, so far as relates to reproductive activity. As regards vegetative activity and general nutrition conditions, no essential difference was observed between the two groups of plants. As the alternations of light and darkness were progressively shortened, increasing evidence of impaired nutrition, including chlorosis, attenuation, and decreased growth, culminated in alternations of about one minute; but with further shortening of the alternations there was a rapid return to approximately normal nutrition and growth. It is evident, therefore, that the effects on reproductive activity were dependent primarily on whether the long-day or short-day type of plant was involved; but effects on nutrition and growth were conditioned chiefly by the specific alternation of light and darkness rather than by the type of plant.

Summarizing the discussion, it will be seen that while development of the contrasted characteristics of the two groups of plants which have been classed as long-day and short-day is based almost entirely on their observed responses to differences in the light period, this mode of classification seems to be a more or less natural one. Certain contrasts in growth characteristics, together with the marked differences with respect to reproductive activity in relation to day length manifested by the two groups,

are in line with their general behavior in nature as well as with their comparative regional and seasonal growth characteristics. In their responses to abnormal light periods the two types also show definite contrast, so far as concerns initiation of reproductive activity, although apparently this does not hold true with respect to maintenance of the general nutrition of the plant. However, any adequate interpretation of the exact significance of these contrasted responses must be left to the future. For those plants which are more sensitive to the length-of-day factor, there exists a fairly definite critical light period which constitutes the dividing line between day lengths favorable to flowering and fruiting and those tending to produce a purely vegetative type of activity. In the short-day group flowering is initiated by day lengths shorter than the critical, and in the long-day group flowering is initiated by day lengths in excess of the critical. Commonly among annuals and herbaceous perennials the alternative vegetative stage of the short-day type is characterized by indeterminate elongation of the axis, while a prominent feature of the vegetative stage in the long-day type is embodied in a leaf-rosette form of growth without significant stem elongation. To determine the classification of a given plant it will usually suffice to observe the nature of the response to a day length less than, and to one greater than the critical light period. The essential characteristic of the less sensitive or indeterminate group of plants is that they possess no clearly defined critical light period.

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EFFECTS OF STARVATION ON DISTRIBUTION OF MINERAL NUTRIENTS IN FRENCH PRUNE TREES GROWN IN CULTURE SOLUTIONS

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(WITH FIFTEEN FIGURES)

Introduction

Leaf samples were taken for analysis, from the entire group of French prune trees starved for various elements (**2**), at three dates during 1930, the second year of starvation. The first collections were on May 15, the second on July 25, and the third and last on November 5. By the time of the last collection, several groups of trees (–Ca, –P, –N) had dropped all of their leaves, and therefore no analyses are reported for leaves of these groups, and for this date.

In most respects, the second year analyses (tables I, II; figs. 1, 2) agree with the analyses of the leaves taken at the close of the 1929 season. Leaves from –K cultures were consistently high (on dry-weight basis) in N and P_2O_5 throughout the entire season of 1930. These leaves were about 30 per cent. above normal in CaO and MgO, but showed only 31 per cent. of normal K_2O on November 5.

The CaO content of leaves starved for *calcium* started at 51 per cent. of normal (on May 15), and fell to 38.6 per cent. normal by July 25. They were low in N and in ash, and high in MgO and K_2O throughout the season.

Leaves starved for *magnesium* showed a MgO content of 0.08 per cent. (24.5 per cent. of normal) on May 15, the MgO content remaining almost constant throughout the season. These leaves were extremely low in CaO; even lower than the CaO content of –Ca leaves on July 25, etc. The N content was very high throughout the season, being 3.85 per cent. of the dry weight on November 5 (which figure represents N in a third cycle of young leaves, the only foliage these trees had at the time). P_2O_5 and ash content were also above normal; K_2O content fell off sharply from the early season to November 5, when only 0.705 per cent. (dry weight) was present.

Leaves starved for *phosphate* showed a low N content on May 15, and only 0.09 per cent. P_2O_5 (19.3 per cent. of normal); P_2O_5 was slightly higher by July 25. The leaves were high in MgO and rather low in CaO throughout the season.

Leaves starved for *sulphur* showed (May 15) 0.068 per cent. (dry weight) SO_3, which was 10.0 per cent. of the normal content. The SO_3 rose slightly by July 25 and then fell back to 0.08 per cent. on November 5.

TABLE I

STARVATION SERIES

COMPOSITION AS PERCENTAGE OF DRY WEIGHT OF GROUPS OF STARVED LEAVES (FRENCH PRUNE TREES, SAMPLES TAKEN DURING SECOND YEAR OF STARVATION)

1: LEAVES COLLECTED MAY 15; 2: JULY 25; 3: NOVEMBER 5

TREATMENT	N			ASH			P_2O_5			CaO			MgO			K_2O			SO_3		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
-K	3.5	2.8	2.7	6.83	8.4	11.0	0.67	0.66	0.66	1.69	2.33	3.16	0.70	0.97	0.349	0.55	0.647	0.507	—	—	—
-K + Na	3.46	2.69	2.56	5.87	7.2	9.57	0.65	0.63	0.60	1.42	2.02	3.03	0.56	0.61	0.558	0.42	0.647	0.447	—	—	—
-Ca	2.79	1.74	—	6.17	8.9	—	0.46	0.36	—	0.46	0.57	—	0.71	0.64	—	1.52	2.19	—	—	—	—
-Mg	3.27	3.24	3.85	9.33	9.2	10.8	0.57	0.45	0.399	0.55	0.38	0.506	0.08	0.081	0.084	2.43	1.58	0.706	—	—	—
$-PO_4$*	2.75	2.63	—	6.66	10.9	—	0.09	0.11	—	1.06	1.29	—	0.53	0.69	—	1.60	1.16	—	—	—	—
$-SO_4$	4.10	2.21	1.62	7.66	9.5	12.2	0.65	0.33	0.24	0.81	1.51	2.23	0.39	0.42	0.391	1.40	1.46	1.71	0.068	0.132	0.08
-N	1.61	1.26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Complete or AZ	3.40	2.53	2.04	8.30	10.3	15.9	0.47	0.39	0.33	0.90	1.50	2.47	0.33	0.43	0.43	1.42	1.05	1.66	0.693	0.810	0.952

* July 25 sample of $-PO_4$ leaves consisted of fallen ones.

TABLE II

STARVATION SERIES

COMPOSITION AS PERCENTAGE OF ASH OF GROUPS OF STARVED LEAVES, SAMPLES TAKEN DURING SECOND YEAR OF STARVATION

1: SAMPLE COLLECTED MAY 15; 2: JULY 25; 3: NOVEMBER 5

TREATMENT	N			ASH			P_2O_5			CaO			MgO			K_2O			SO_3		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
–K	3.5	2.8	2.7	6.83	8.4	11.0	9.8	7.8	6.0	24.7	27.1	28.7	10.2	11.6	3.16	8.1	6.74	4.6	—	—	—
–K + Na	3.46	2.69	2.56	5.87	7.2	9.57	11.1	8.7	6.2	24.1	27.5	30.4	9.52	8.4	5.8	7.15	7.90	4.67	—	—	—
–Ca	2.79	1.74	—	6.17	8.9	—	7.5	4.0	—	7.4	6.4	—	12.0	7.2	—	24.0	24.8	—	—	—	—
–Mg	3.27	3.24	3.85	9.33	9.2	10.8	6.1	4.9	3.7	8.6	4.2	4.6	0.830	0.92	0.77	25.1	17.4	6.52	—	—	—
$-PO_4$*	2.75	2.63	—	6.66	10.9	—	1.35	1.02	—	16.0	11.8	—	8.00	6.3	—	24.0	10.6	—	—	—	—
$-SO_4$	4.10	2.21	1.62	7.66	9.5	12.2	8.5	3.47	4.9	10.6	14.5	18.3	5.1	4.5	3.2	18.2	15.6	14.0	0.88	1.39	0.65
Complete or AZ	3.40	2.53	2.04	8.30	10.3	15.9	5.6	3.78	2.07	12.1	15.2	15.7	4.2	4.4	2.7	17.1	10.2	10.5	8.3	7.8	6.06

* July 25 sample of –P leaves consisted of fallen ones.

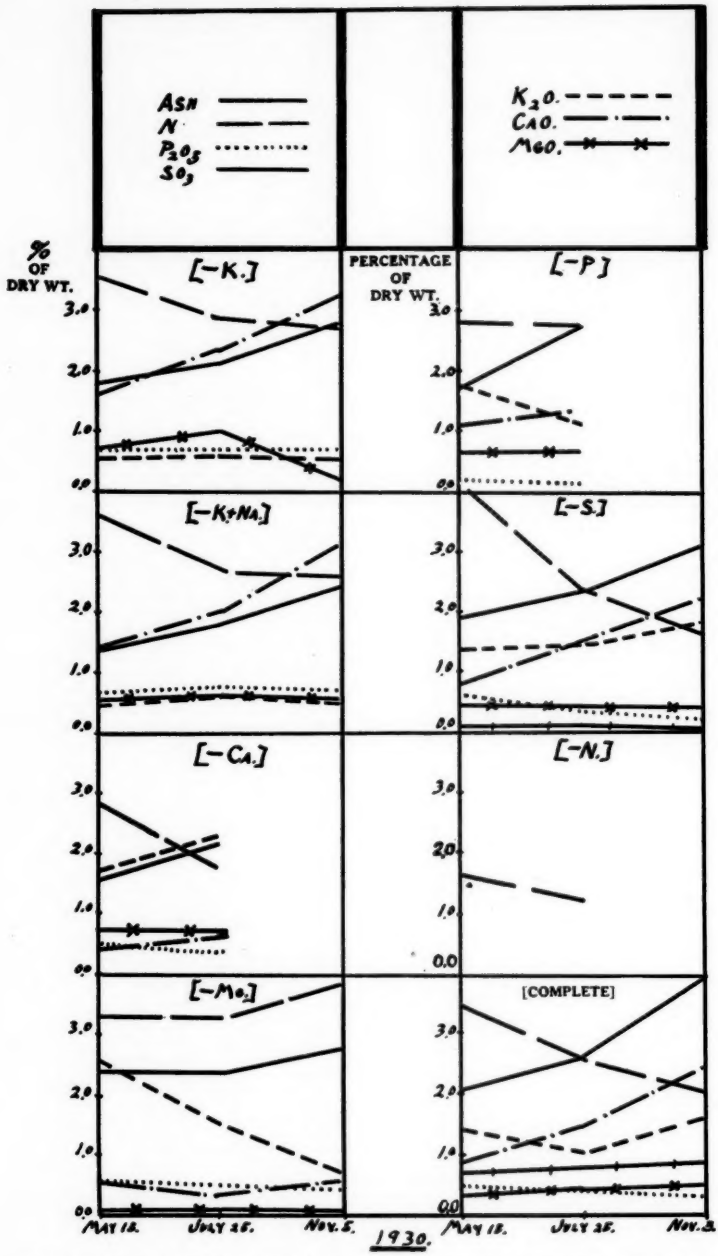

FIG. 1. Seasonal variation in the mineral composition of groups of French prune leaves starved for various elements for the two-year period 1929–1930; samples taken on three dates during the second year of starvation.

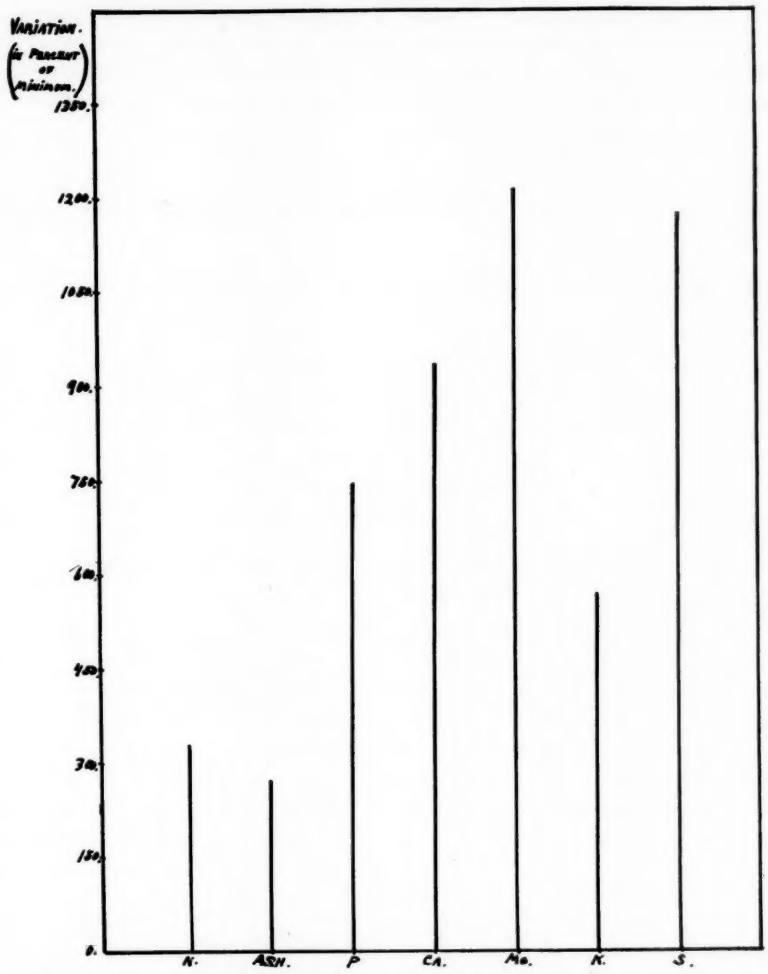

FIG. 2. Maximum variability of the mineral constituents (on percentage dry weight basis, the minimum in each case being taken as equal to 100 per cent.) obtained in starvation treatments, with French prune tree leaves taken in the second year of starvation.

The N content of –S leaves on May 15 was 4.7 per cent., the extreme upper limit of N content recorded anywhere in the experiment. The N fell rapidly to a low level of 1.62 per cent. by November 5.

Leaves starved for *nitrogen* showed 1.61 per cent. N on May 15 and 1.26 per cent. on July 25. No ash analyses were made of this group.

"Complete" (AZ) leaves showed 3.4 per cent. N at the first collection; 2.53 per cent. on July 25; and 2.04 per cent. on November 5. These leaves appeared to be below normal in K_2O throughout the season, although K was always present in the culture solution. They were slightly low in CaO content; otherwise the analyses checked fairly well with well-grown orchard leaves of similar trees.

The *maximum* (in percentage based on the minimum as = 100) content of particular elements, on a dry-weight basis produced by starvation methods in these leaves, was:

	PER CENT.
For total N	325
" " Ash	270
" " P_2O_5	744
" " CaO	831
" " MgO	1212
" " K_2O	566
" " SO_3	1180

Inorganic composition of bark, wood, and root at end of two years' starvation

After the eighty French prune trees of the starvation experiment had completed two years of growth in culture solutions, the trees were harvested, washed, weighed, and separated into six divisions: young (one and two-year) roots; main (three-year) roots; trunk (three-year) wood; trunk bark; young (one and two-year) branch wood; and young branch bark. All tissues were dried, ground, and analyzed for N, Mg, Ca, P, K, and Fe. Nitrogen was obtained by the Kjeldahl method, potassium by the platinic chloride (gravimetric), calcium by the volumetric ($KMnO_4$ titration), magnesium by precipitation with ammonium phosphate, phosphorus by the Denigés colorimetric, and iron by the thiocyanate method.

The five trees taken from the group at the start of the experiment were separated into three divisions: trunk bark, trunk wood, and root, the last corresponding to the main root section of the starved trees harvested two years later.

In the original tree tissue (at the start of the experiment) the ash content of the top trunk bark was 6.36 per cent. of the dry weight; the ash content of the wood was 0.8 per cent.; and in the root (bark plus wood) it was 2.0 per cent. The roots were very high in phosphorus; the calcium and potassium contents were about equal; and most of the iron stored in the tree was in the root. The "original" trees, then, were moderately well

TABLE III

MINERAL CONSTITUENTS IN ORIGINAL TREE TISSUES AT START OF EXPERIMENT; IN PERCENTAGE OF DRY WEIGHT

	ASH	NITROGEN	P_2O_5	K_2O	SO_3	CaO	MgO	Fe_2O_3
	%	%	%	%	%	%	%	%
Top, Trunk, Bark	6.36	1.8	0.34	1.82	0.3	1.97	0.15	0.055
Top, Trunk, Wood	0.80	0.75	0.07	0.39	0.02	0.15	0.048	0.0014
Root	2.00	1.10	0.24	0.70	0.09	0.70	0.087	0.046

stocked with the more common elements necessary for growth. Table IIIA gives the results on the basis of dry-weight yield for the entire tree.

TABLE IIIA

YIELD PER TREE OF DRY WEIGHT, ASH, AND NITROGEN, AFTER TWO YEARS' TREATMENT (STARVATION SERIES)

TREE	DRY WEIGHT	TOTAL ASH PER TREE	TOTAL N
	gm.	*gm.*	*gm.*
Complete	348.2	10.80	5.050
−S	200.2	6.30	3.300
−P	131.5	4.01	1.150
−K+Na	119.2	3.44	1.390
−Ca	98.3	2.32	0.710
−N	95.8	2.54	0.340
−K	92.4	2.79	1.048
−Mg	91.1	3.45	0.854
Original	53.3	1.01	0.540

Inorganic composition of trees, taking whole tree as a unit

It is interesting to note that the *Mg-starved* trees were lowest of the series in dry-weight yield, but ranked fourth in total ash per tree, and sixth in total nitrogen. Mg-starved tissues throughout the tree were found to be high in ash content (the highest of any group of trees).

Trees starved for *potassium* showed (tables IV–IX; figs. 3–10) low N (percentage dry weight, compared with that of check trees), slightly lower ash content, high P_2O_5, normal MgO, and CaO equal to twice that of check trees. K_2O was equal to 40 per cent. of normal.

Similar composition was found in the −K + Na trees, but with somewhat higher N content, lower P_2O_5, and much lower ash and CaO content than

TABLE IV

TOTAL NITROGEN CONTENT AND ASH CONTENT AS PERCENTAGES OF DRY WEIGHT IN PARTS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25, 1930		BARK (1 AND 2 YEARS)		BARK TRUNK (3 YEARS)		WOOD (1 AND 2 YEARS)		WOOD TRUNK (3 YEARS)		ROOTS (1 AND 2 YEARS)		ROOTS MAIN (3 YEARS)	
	N	ASH	N	ASH	N	ASH	N	ASH	N	ASH	N	ASH	N	ASH
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
–K	2.8	8.4	1.6	6.9	1.14	6.1	0.7	0.8	0.25	0.55	2.05	4.1	1.37	3.0
–K + Na	2.69	7.23	1.51	5.6	1.22	7.8	0.605	1.15	0.24	0.77	2.31	4.15	1.63	2.66
–Ca	1.74	8.9	1.04	4.0	1.02	4.5	——	1.2	0.15	0.60	1.47	3.85	0.91	2.6
–Mg	3.24	9.2	1.52	8.4	0.78	8.6	0.52	1.6	0.12	0.70	1.88	6.55	1.13	3.86
$-NO_3$	1.26	.....	0.62	4.4	0.51	5.0	0.36	1.63	0.09	0.62	0.77	4.55	0.32	2.8
$-PO_4$	2.63	10.9	1.25	5.3	1.09	5.8	0.44	1.33	0.15	0.70	1.68	4.35	0.88	3.1
$-SO_4$	2.21	9.5	2.31	5.7	2.10	4.7	0.47	0.92	0.36	0.52	2.49	5.15	1.90	2.7
Complete	2.53	10.3	1.57	4.9	1.35	6.4	0.67	0.80	0.31	0.55	2.68	5.05	1.70	2.6
			Top 12		Top trunk									
Distilled H_2O	.....	.....	0.27	3.1	0.24	2.5	.....	.....	.....	.....	1.4	3.8	0.33	2.2

TABLE V

P_2O_5 AS PERCENTAGE OF ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PARTS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK		WOOD (1 AND 2 YEARS)		WOOD TRUNK		ROOT (1 AND 2 YEARS)		ROOT MAIN (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
– K	7.8	0.66	6.6	0.455	5.4	0.329	22.5	0.180	43.6	0.24	9.5	0.389	12.5	0.375
– K + Na	8.7	0.63	8.5	0.476	4.6	0.358	17.3	0.198	25.3	0.19	9.7	0.397	14.4	0.384
– Ca	4.0	0.36	11.7	0.468	6.6	0.297	.....	.....	21.0	0.127	9.6	0.364	10.4	0.270
– Mg	4.9	0.45	6.7	0.562	2.1	0.180	8.7	0.149	9.6	0.067	10.3	0.669	8.6	0.336
– NO_3	.....	.....	8.8	0.388	4.4	0.220	9.2	0.139	20.5	0.127	12.4	0.558	7.7	0.213
– PO_4	1.02	0.112	5.1	0.270	1.5	0.087	3.8	0.0494	6.4	0.045	2.8	0.120	3.3	0.101
– SO_4	3.47	0.33	7.0	0.402	7.02	0.329	15.2	0.139	15.9	0.082	9.4	0.479	11.2	0.303
Complete	3.78	0.39	9.2	0.450	5.10	0.326	18.7	0.148	23.0	0.127	7.4	0.370	14.2	0.384
			Top 12		Top trunk									
Distilled H_2O	.....	.....	8.7	0.270	4.7	0.117	.....	.....	.....	.....	11.8	0.448	8.5	0.193

TABLE VI

K_2O IN PERCENTAGE ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PORTIONS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK (3 YEARS)		WOOD (1 AND 2 YEARS)		WOOD TRUNK (3 YEARS)		ROOT (1 AND 2 YEARS)		ROOT MAIN OR TRUNK (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
−K	6.74	0.647	8.2	0.565	9.8	0.597	15.6	0.125	11.8	0.066	8.8	0.360	8.8	0.264
−K + Na	7.9	0.647	9.3	0.510	7.6	0.592	9.1	0.104	18.0	0.138	8.4	0.344	10.0	0.266
−Ca	24.8	2.19	26.9	1.070	25.4	1.140	35.6	0.430	44.0	0.264	33.2	1.26	31.5	0.810
−Mg	17.4*	1.58	16.1	1.356	17.2	1.460	22.8	0.364	47.8	0.333	22.8	1.48	20.0	0.772
$-NO_3$	.....	.....	21.5	0.946	23.7	1.180	14.4	0.234	33.6	0.208	26.3	1.16	22.9	0.641
$-PO_4$	10.6	1.16	19.0	1.000	24.4	1.410	17.0	0.226	30.0	0.210	19.3	0.829	17.4	0.539
$-SO_4$	15.6	1.46	21.7	1.020	17.4	0.990	17.3	0.159	30.1	0.156	12.0	0.612	24.2	0.653
Complete	10.2	1.05	20.0	0.980	17.2	1.100	28.1	0.225	30.0	0.165	18.4	0.920	22.6	0.587
			Top 12		Top trunk									
Distilled H_2O	.....	.....	16.1	0.499	16.4	0.410	.....	.....	.....	.....	13.3	0.505	13.2	0.288

TABLE VII

CaO IN PERCENTAGE OF ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PORTIONS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK		WOOD (1 AND 2 YEARS)		WOOD TRUNK (3 YEARS)		ROOT (1 AND 2 YEARS)		ROOT MAIN (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
– K	27.1	2.33	31.6	2.18	48.8	2.97	24.2	0.193	28.4	0.156	27.7	1.13	30.4	0.912
– K + Na	27.5	2.02	43.8	2.45	35.8	2.79	16.0	0.184	16.7	0.128	13.3	0.54	22.3	0.580
– Ca	6.4	0.57	6.6	0.26	17.3	0.78	9.4	0.113	19.2	0.115	4.4	0.167	12.4	0.322
– Mg	4.2	0.38	36.5	3.06	41.6	3.57	21.5	0.344	28.3	0.198	20.5	1.332	29.9	1.130
– NO_3	.....	.....	23.6	1.148	28.0	1.40	10.6	0.173	28.8	0.178	4.0	0.183	17.0	0.476
– PO_4	11.8	1.29	30.2	1.60	37.9	2.20	18.4	0.244	22.5	0.157	11.3	0.486	22.1	0.685
– SO_4	14.5	1.51	31.1	1.49	39.8	2.19	19.4	0.178	28.1	0.146	9.3	0.474	14.8	0.399
Complete	15.2	1.50	25.0	1.22	29.7	1.90	14.3	0.114	26.0	0.143	6.7	0.335	10.5	0.273
			Top 12		Top trunk									
Distilled H_2O	.....	.....	15.2	0.480	15.6	0.380	.....	.....	.....	.....	9.1	0.340	13.7	0.310

TABLE VIII

MgO AS PERCENTAGE OF ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PORTIONS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK		WOOD (1 AND 2 YEARS)		WOOD TRUNK		ROOT (1 AND 2 YEARS)		ROOT MAIN (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
– K	11.6	0.97	4.4	0.303	5.9	0.359	4.0	0.032	2.1	0.0115	3.3	0.135	5.1	0.153
– K + Na	8.4	0.61	4.71	0.262	4.4	0.342	3.0	0.034	2.11	0.0161	4.8	0.196	5.7	0.148
– Ca	7.24	0.64	4.8	0.192	6.7	0.301	7.3	0.088	3.0	0.018	6.95	0.262	6.7	0.174
– Mg	0.92	0.081	1.2	0.100	0.87	0.074	1.6	0.025	1.32	0.009	0.96	0.062	2.0	0.076
– NO_3	.....	.....	7.6	0.343	4.1	0.205	3.2	0.052	3.7	0.022	1.84	0.081	3.6	0.100
– PO_4	6.3	0.697	5.7	0.302	4.5	0.261	2.6	0.034	2.4	0.016	3.40	0.146	3.7	0.114
– SO_4	4.5	0.425	4.3	0.259	6.3	0.356	3.8	0.036	4.5	0.0234	4.40	0.224	5.5	0.148
Complete	4.4	0.43	4.2	0.205	4.2	0.268	3.7	0.029	3.8	0.0209	3.57	0.178	5.6	0.145
			Top 12		Top trunk									
Distilled H_2O	.....	.....	7.6	0.235	5.6	0.140	.....	.....	.....	.....	4.7	0.178	6.1	0.134

TABLE IX

Fe_2O_3 AS PERCENTAGE OF ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PARTS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK (3 YEARS)		WOOD (1 AND 2 YEARS)		WOOD TRUNK (3 YEARS)		ROOT (1 AND 2 YEARS)		ROOT MAIN (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
– K	0.78	0.065	0.48	0.030	0.40	0.024	2.34	0.016	0.85	0.0047	8.8	0.360	3.2	0.096
– K + Na	1.20	0.086	0.68	0.038	0.49	0.038	1.90	0.021	1.02	0.0078	9.0	0.373	3.22	0.083
– Ca	1.40	0.126	1.091	0.046	0.89	0.040	0.66	0.007	0.78	0.0046	3.7	0.142	1.70	0.044
– Mg	2.40*	0.220	0.38	0.032	0.70	0.060	1.12	0.017	1.21	0.0084	6.7	0.435	1.51	0.058
– NO_3	.....	.....	0.78	0.034	1.30	0.065	0.86	0.014	0.86	0.0053	3.4	0.156	1.02	0.028
– PO_4	2.0	0.218	0.87	0.046	0.77	0.044	0.56	0.0073	0.87	0.0061	6.8	0.292	0.87	0.027
– SO_4	1.2	0.114	0.62	0.024	0.43	0.029	1.90	0.018	1.37	0.0071	3.3	0.170	1.06	0.028
Complete	1.56	0.161	0.49	0.024	0.39	0.025	1.12	0.0088	1.24	0.0068	2.3	0.105	0.72	0.0187
Original tissue	.....	.....	.....	.....	0.85	0.055	.....	.....	0.175	0.0014	.....	.....	2.30	0.0460
			Top 12		Top trunk									
Distilled H_2O	.....	.....	0.98	0.031	0.77	0.019	.....	.....	.....	.....	0.61	0.023	0.80	0.018

TABLE X

DISTRIBUTION (AVERAGED) OF DRY WEIGHT AND MINERAL CONSTITUENTS

TREATMENT	MATERIAL	DRY WEIGHT		TOTAL N		P_2O_5	
		GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL
		gm.	%	*gm.*	%	*gm.*	%
−K	Wood 1, 2	3.6	3.8	0.025	2.31	0.006	1.9
	Wood trunk	25.3	27.3	0.063	6.10	0.0607	19.7
	Bark 1, 2	6.7	7.3	0.107	10.1	0.0305	10.0
	Bark trunk	9.8	10.8	0.1117	10.6	0.0320	10.4
	Root 1, 2	14.4	15.5	0.2952	28.3	0.0559	18.2
	Root trunk	32.6	35.3	0.4466	42.6	0.1222	39.8
Total		92.4	100.0	1.0485	100.0	0.3073	100.0
Tree average		.....	.....	1.13%	.....	0.33%	.....
−K + Na	Wood 1, 2	7.1	6.0	0.043	3.0	0.014	3.7
	Wood trunk	38.7	32.5	0.093	6.6	0.075	20.0
	Bark 1, 2	7.5	6.3	0.113	8.1	0.035	9.4
	Bark trunk	11.9	10.0	0.145	10.4	0.041	11.3
	Root 1, 2	17.7	14.8	0.408	29.4	0.070	18.7
	Root trunk	36.2	30.4	0.590	42.5	0.139	36.6
Total		119.2	100.0	1.392	100.0	0.3769	100.0
Tree average		.....	.....	1.17%	.....	0.31%	.....
−Ca*	Wood 1, 2	4.6*	4.7	0.023	3.2	0.0068	2.8
	Wood trunk	30.2	30.7	0.0458	6.2	0.0383	16.1
	Bark 1, 2	5.1	5.5	0.053	7.4	0.0238	10.0
	Bark trunk	14.0	14.2	0.1428	20.0	0.0415	17.4
	Root 1, 2	8.0	8.2	0.1176	16.5	0.0291	12.3
	Root trunk	36.4	37.0	0.3312	46.6	0.0983	41.4
Total		98.3	100.0	0.7129	100.0	0.2378	100.0
Tree average		.....	.....	0.727%	.....	0.24%	.....
−Mg	Wood 1, 2	5.5	6.04	0.0286	3.5	0.0076	2.9
	Wood trunk	29.5	32.4	0.0354	4.4	0.0197	7.7
	Bark 1, 2	7.8	8.6	0.1170	14.7	0.0438	17.1
	Bark trunk	8.3	9.1	0.0647	8.0	0.0149	5.8
	Root 1, 2	10.8	11.8	0.2030	27.5	0.0722	28.2
	Root trunk	29.2	32.1	0.3299	42.1	0.0981	38.3
Total		91.1	100.0	0.7786	100.0	0.2503	100.0
Tree average		.....	.....	0.854%	.....	0.280%	.....

TABLE X

THROUGHOUT THE TREE (FRENCH PRUNE) AFTER TWO YEARS' TREATMENT

Ash		CaO		MgO		K_2O		Fe_2O_3	
Gross	Total	Gross	Total	Gross	Total	Gross	Total	Gross	Total
gm.	%	*gm.*	%	*gm.*	%	*gm.*	%	*gm.*	%
0.0288	1.0	0.0067	0.7	0.0011	0.9	0.0047	1.8	0.00057	0.6
0.1391	4.9	0.0394	4.3	0.0029	2.2	0.0167	6.5	0.00119	1.4
0.4623	16.5	0.1460	15.4	0.0203	16.4	0.0378	14.8	0.00280	3.1
0.5978	21.3	0.2910	30.8	0.0351	27.2	0.0585	22.9	0.00235	2.5
0.5904	21.1	0.1627	17.3	0.0194	15.1	0.0518	20.3	0.0518	57.4
0.9780	34.9	0.2973	31.5	0.0498	38.2	0.0860	33.7	0.0313	35.0
2.7964	100.0	0.9431	100.0	0.1286	100.0	0.2555	100.0	0.09000	100.0
3.02%	.....	1.02%	.....	0.13%	.....	0.27%	.....	0.098%	.....
0.0816	2.5	0.013	1.4	0.0024	1.5	0.0073	2.2	0.0015	1.4
0.297	8.4	0.049	5.6	0.0062	3.9	0.0530	16.2	0.0029	2.8
0.420	12.4	0.1837	20.7	0.0196	12.4	0.0380	11.6	0.0028	2.7
0.928	27.0	0.3320	37.5	0.0408	26.0	0.0704	21.8	0.0045	4.1
0.734	21.4	0.0964	10.9	0.0347	22.1	0.0608	18.6	0.0660	61.1
0.962	28.3	0.2099	23.9	0.0535	34.1	0.0963	29.6	0.0300	27.9
3.446	100.0	0.8845	100.0	0.1572	100.0	0.3264	100.0	0.1077	100.0
2.65%	.....	0.74%	.....	0.132%	.....	0.27%	.....	0.090%	.....
0.0556	2.3	0.00519	1.7	0.0040	2.7	0.0197	2.7	0.0003	0.8
0.1812	7.7	0.0347	11.8	0.0054	3.7	0.0797	11.2	0.00144	3.9
0.2065	8.8	0.01341	4.6	0.0097	6.8	0.0545	7.6	0.00224	6.0
0.6300	27.1	0.1089	37.3	0.0421	29.3	0.1596	22.4	0.0056	15.1
0.3080	13.3	0.0133	4.6	0.0209	14.7	0.1008	14.2	0.0113	30.7
0.9464	40.8	0.1172	40.0	0.0633	43.8	0.2970	41.9	0.0160	43.5
2.3277	100.0	0.2926	100.0	0.1454	100.0	0.7113	100.0	0.0368	100.0
2.37%	.....	0.298%	.....	0.14%	.....	0.72%	.....	0.037%	.....
0.088	2.5	0.0189	1.7	0.0013	2.9	0.020	2.7	0.00093	1.2
0.2065	5.9	0.0584	5.3	0.0025	5.5	0.098	13.4	0.00247	3.3
0.6552	18.9	0.2386	22.1	0.0078	16.5	0.105	14.3	0.00248	3.3
0.7138	20.7	0.2963	27.3	0.0061	13.2	0.1211	16.7	0.0049	6.4
0.6620	19.2	0.1427	13.1	0.0067	14.5	0.1598	21.9	0.0469	63.2
0.1271	32.0	0.3299	30.5	0.0222	47.4	0.2254	31.0	0.0169	22.6
3.4526	100.0	1.0848	100.0	0.0467	100.0	0.7298	100.0	0.0744	100.0
3.79%	.....	1.19%	.....	0.05%	.....	0.80%	.....	0.081%	.....

TABLE X (*Continued*)

DISTRIBUTION (AVERAGED) OF DRY WEIGHT AND MINERAL CONSTITUENTS

TREATMENT	MATERIAL	DRY WEIGHT		TOTAL N		P_2O_5	
		GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL
		gm.	%	*gm.*	%	*gm.*	%
$-NO_3$	Wood 1, 2	0.8	0.83	0.0028	0.8	0.0012	0.5
	Wood trunk	31.7	33.10	0.0285	8.4	0.0402	16.5
	Bark 1, 2	1.3	1.35	0.0080	2.3	0.0050	2.0
	Bark trunk	9.6	10.02	0.1439	42.4	0.1043	42.8
	Root 1, 2	18.7	19.50	0.0489	14.4	0.0211	8.7
	Root trunk	33.7	35.20	0.1078	31.7	0.0717	29.5
Total		95.8	100.0	0.3399	100.0	0.2435	100.0
Tree average		.....	.....	0.35%	.....	0.253%	.....
$-PO_4$	Wood 1, 2	15.3	11.6	0.0673	5.8	0.0075	5.4
	Wood trunk	29.3	22.3	0.0439	3.8	0.0132	9.4
	Bark 1, 2	15.7	12.0	0.1962	17.0	0.0424	31.0
	Bark trunk	9.7	7.4	0.1057	9.1	0.0084	6.0
	Root 1, 2	24.5	18.5	0.4116	35.8	0.0294	22.0
	Root trunk	37.0	28.2	0.3256	28.5	0.0373	27.0
Total		131.5	100.0	1.1502	100.0	0.1382	100.0
Tree average		.....	.....	0.88%	.....	0.105%	.....
$-S$	Wood 1, 2	14.8	7.4	0.0695	2.1	0.0203	3.4
	Wood trunk	47.4	23.7	0.1706	5.1	0.0388	6.5
	Bark 1, 2	16.2	8.1	0.4050	12.2	0.0533	9.0
	Bark trunk	19.8	9.9	0.4177	12.6	0.0796	13.4
	Root 1, 2	51.9	25.9	1.292	39.0	0.2486	41.9
	Root trunk	50.1	25.0	0.952	29.0	0.1518	25.8
Total		200.2	100.0	3.305	100.0	0.5926	100.0
Tree average		.....	.....	1.65%	.....	0.296%	.....
Complete	Wood 1, 2	39.0	11.2	0.261	5.1	0.0581	5.7
	Wood trunk	81.8	23.5	0.253	5.0	0.1038	10.3
	Bark 1, 2	41.0	11.7	0.643	12.8	0.1845	18.4
	Bark trunk	31.3	9.0	0.422	8.3	0.0717	7.1
	Root 1, 2	83.1	23.8	2.227	44.4	0.3074	30.7
	Root trunk	72.0	20.8	1.224	24.4	0.2764	27.8
Total		348.2	100.0	5.030	100.0	1.0019	100.0
Tree average		.....	.....	1.44%	.....	0.287%	.....
Original tissue	Top wood	23.5	44.0	0.176	32.5	0.016	18.0
	Top bark	5.31	10.0	0.095	17.5	0.018	20.0
	Root trunk	24.5	46.0	0.269	50.0	0.059	62.0
Total		53.3	100.0	0.540	100.0	0.093	100.0
Tree average		.....	.....	1.01%	.....	0.175%	.....

TABLE X (*Continued*)

THROUGHOUT THE TREE (FRENCH PRUNE) AFTER TWO YEARS' TREATMENT

Ash		CaO		MgO		K_2O		Fe_2O_3	
Gross	Total	Gross	Total	Gross	Total	Gross	Total	Gross	Total
gm.	%	*gm.*	%	*gm.*	%	*gm.*	%	*gm.*	%
0.013	0.5	0.0013	0.3	0.00041	0.5	0.00187	0.3	0.00011	0.2
0.1965	7.7	0.0564	14.0	0.0069	8.6	0.0659	10.5	0.00168	3.5
0.0572	2.2	0.0149	3.7	0.0044	5.5	0.0123	1.9	0.0004	0.8
0.4800	18.8	0.1344	33.5	0.0196	24.5	0.1132	18.1	0.0062	13.4
0.8508	33.5	0.0342	8.5	0.0151	18.9	0.2174	34.7	0.0291	61.9
0.9436	37.3	0.1604	40.0	0.0337	42.0	0.2160	34.5	0.0094	20.2
2.5411	100.0	0.4016	100.0	0.0801	100.0	0.6263	100.0	0.0468	100.0
2.64%	.....	0.417%	.....	0.083%	.....	0.65%	.....	0.048%	.....
0.2035	5.0	0.0376	4.0	0.0052	3.2	0.0345	4.3	0.0014	1.1
0.2051	5.1	0.0460	5.0	0.0049	3.0	0.0615	7.7	0.0017	1.7
0.8321	20.7	0.2512	27.2	0.0474	29.4	0.1570	19.8	0.0092	9.4
0.5626	14.0	0.2134	23.1	0.0253	15.6	0.1367	17.3	0.0042	4.3
1.0657	26.6	0.1190	12.9	0.0357	22.4	0.2031	25.6	0.0715	73.5
1.1479	28.6	0.2534	27.8	0.0422	26.4	0.1994	25.3	0.0099	10.0
4.0160	100.0	0.9203	100.0	0.1607	100.0	0.7922	100.0	0.0976	100.0
3.06%	.....	0.70%	.....	0.122%	.....	0.604%	.....	0.074%	.....
0.1361	2.1	0.0263	2.1	0.0054	1.7	0.0235	2.1	0.0026	2.2
0.2464	3.9	0.0692	5.7	0.0111	3.5	0.0739	6.7	0.0033	2.8
0.761	12.1	0.2414	20.0	0.0333	10.7	0.1652	15.0	0.0047	4.0
1.138	10.1	0.4296	35.4	0.0711	22.9	0.1960	17.8	0.0047	4.0
2.673	42.4	0.2460	20.3	0.1162	37.3	0.3176	28.8	0.0882	75.2
1.352	21.4	0.1998	16.5	0.0741	23.9	0.3271	29.6	0.0140	11.8
6.307	100.0	1.2123	100.0	0.3112	100.0	1.1033	100.0	0.1175	100.0
3.15%	.....	0.606%	.....	0.155%	.....	0.55%	.....	0.058%	.....
0.312	2.9	0.0473	2.7	0.0113	2.6	0.0877	4.0	0.0034	2.6
0.449	4.1	0.1169	6.7	0.0163	3.6	0.1349	6.3	0.0055	4.3
2.009	18.6	0.5002	28.9	0.0820	18.4	0.4018	18.6	0.0098	7.7
2.003	18.5	0.5947	34.3	0.0838	18.5	0.3443	15.9	0.0078	6.2
4.155	38.6	0.2783	16.1	0.1479	31.8	0.7728	35.7	0.0872	68.6
1.872	17.3	0.1965	11.3	0.1044	22.1	0.4226	19.5	0.0134	10.6
10.800	100.0	1.7339	100.0	0.4457	100.0	2.1641	100.0	0.1271	100.0
3.10%	.....	0.49%	.....	0.13%	.....	0.62%	.....	0.036%	.....
0.188	18.6	0.035	11.3	0.0113	28.2	0.091	25.8	0.0003	2.0
0.337	33.2	0.104	33.3	0.0079	19.3	0.096	26.5	0.0029	20.4
0.490	48.2	0.171	55.2	0.0213	52.5	0.171	47.7	0.011	7.76
1.015	100.0	0.310	100.0	0.040	100.0	0.358	100.0	0.0142	100.0
1.91%	.....	0.58%	.....	0.075%	.....	0.675%	.....	0.0268%	.....

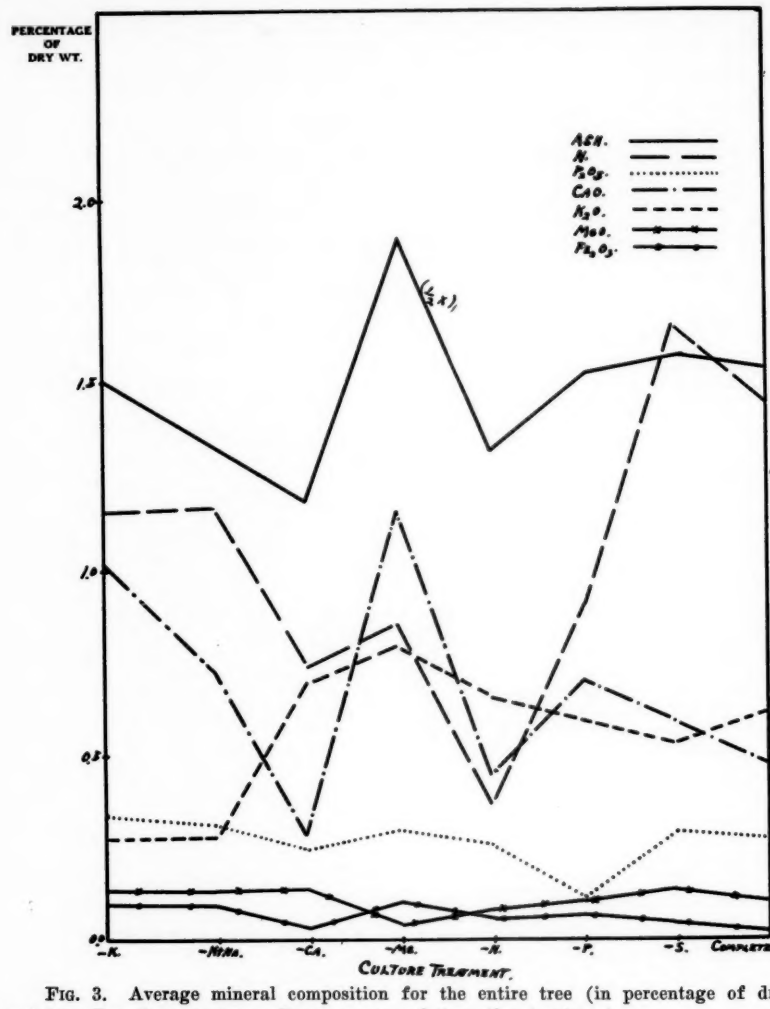

FIG. 3. Average mineral composition for the entire tree (in percentage of dry weight); French prune trees after two years of starvation treatment.

in the –K trees. MgO and K_2O contents were almost identical in the two groups.

Trees in the *minus-Ca* group had only one-half as high N (on per cent. dry weight) as had the complete-solution trees. P_2O_5 was slightly low, ash was very low, CaO was 60 per cent. of the check (or normal), MgO was slightly above normal, and K_2O was about 16 per cent. above normal.

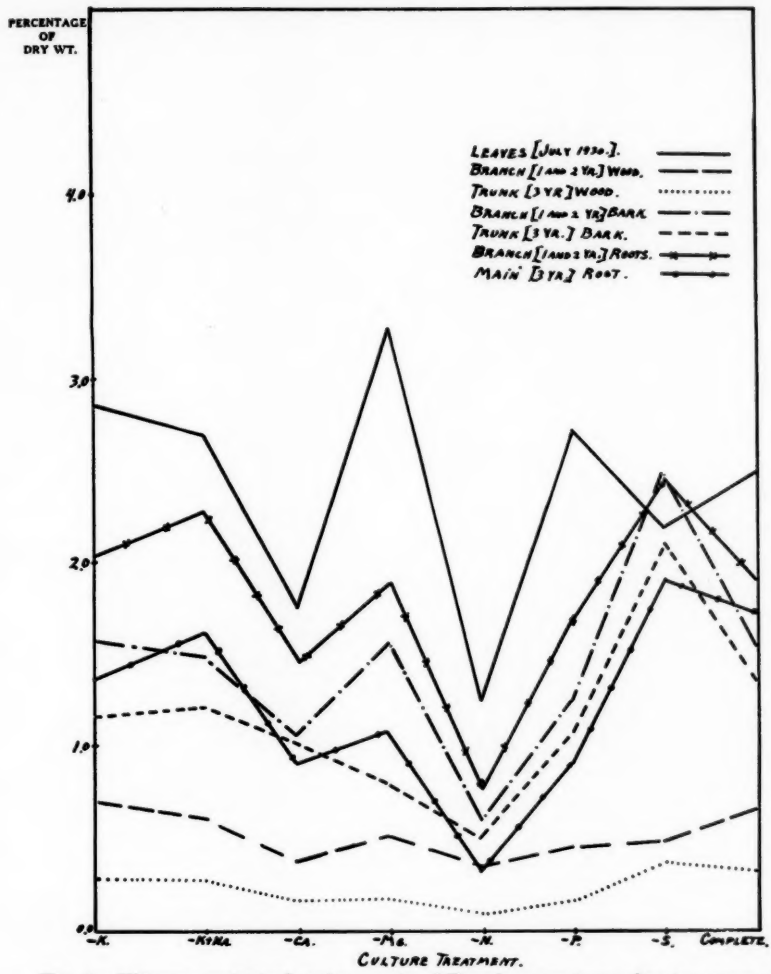

FIG. 4. Nitrogen content of various parts of French prune trees after two years of starvation for given elements.

Trees *starved for N* showed a content of N equal to 25 per cent. normal; P_2O_5, CaO, and ash lower than normal; MgO far below and K_2O slightly above normal.

Trees *starved for Mg* had 59 per cent. of normal N content, very unevenly distributed throughout the tree, a normal P_2O_5 content, CaO more than twice normal, MgO equal to 38 per cent. normal, and K_2O equal to 30 per cent. above normal.

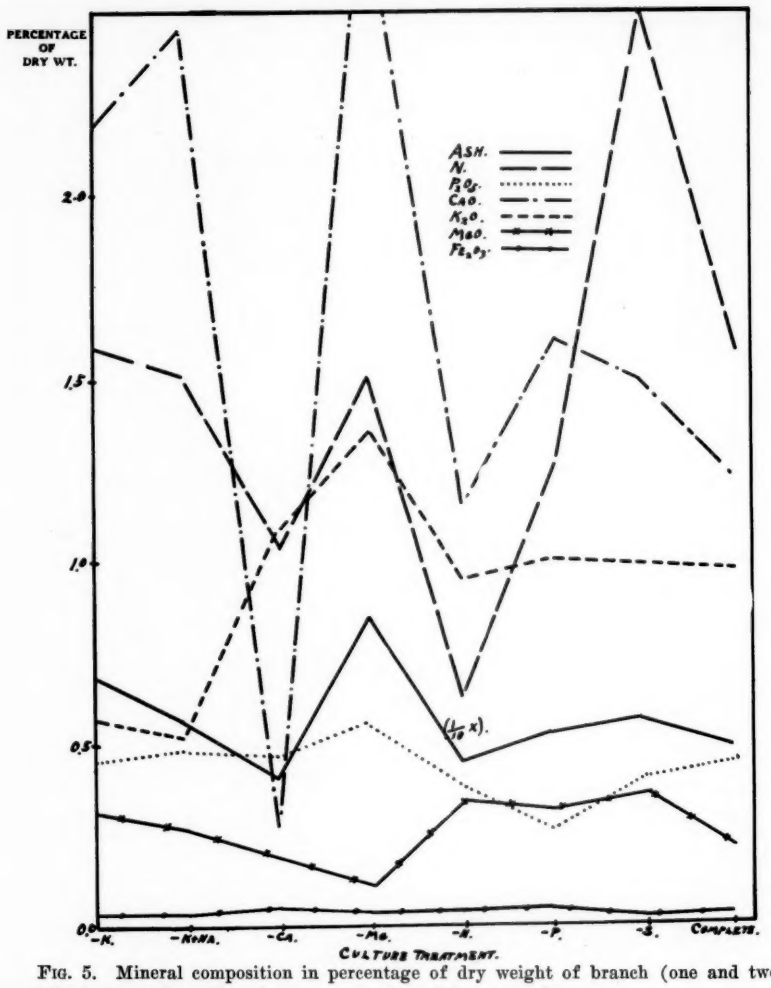

FIG. 5. Mineral composition in percentage of dry weight of branch (one and two year) bark tissues from French prune trees starved for given elements.

The N content of *–P* trees was only 61 per cent. normal, P_2O_5 was 36 per cent. normal, ash was normal, CaO was 40 per cent. above normal, and MgO and K_2O were both somewhat lower than in the complete-solution trees.

Minus-S trees showed the highest N content of any trees in the experiment, being 14 per cent. above normal; P_2O_5 was slightly above normal, as was also ash content, CaO, and MgO; K_2O was about 11 per cent. below normal.

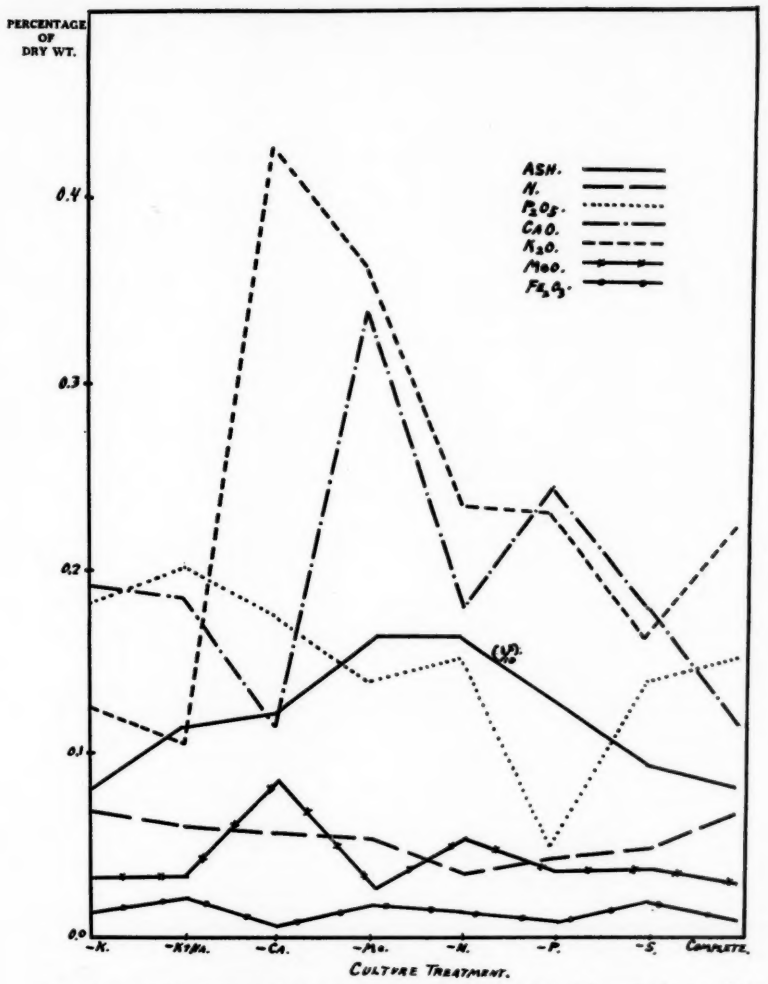

FIG. 6. Mineral composition in percentage of dry weight of branch (one and two year) wood from French prune trees starved for given elements.

Contrasting the analyses of the *complete* trees with those of original trees, we see that during the experiment these trees had gained in percentage N, P_2O_5, ash, MgO, and lost in CaO and K_2O. It must be recalled, however, that the "original tree tissue" aliquot did not include the young tips of either top or root, as these young parts were pruned off before sampling

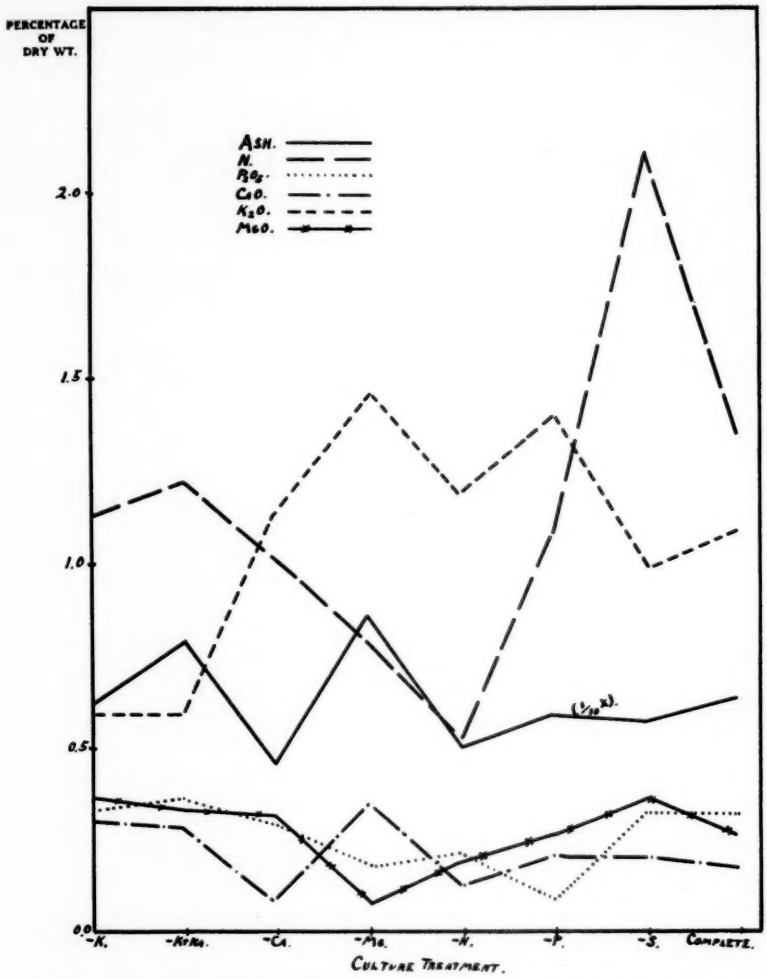

FIG. 7. Mineral composition in percentage of dry weight of trunk (three year) bark tissues from French trees starved for given elements.

was begun. Consequently the two sets of tissues are not entirely comparable. In the following section, parts of the trees (trunk and main root sections) that are entirely comparable, are discussed in detail.

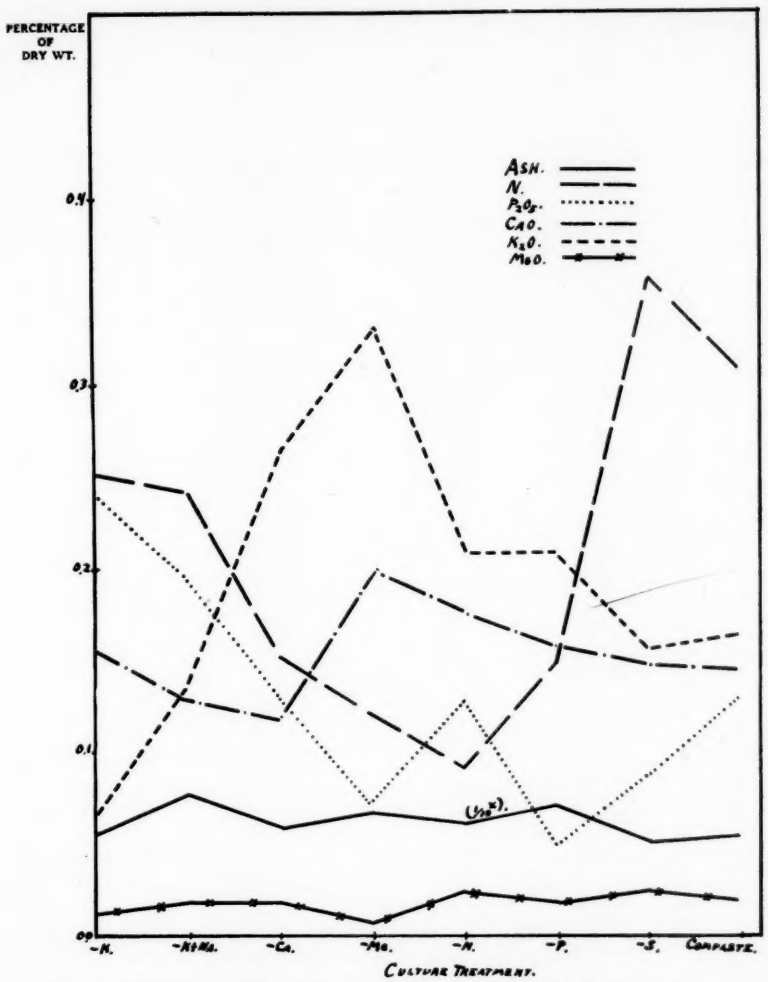

FIG. 8. Mineral composition in percentage of dry weight of trunk (three year) wood from French prune trees starved for given elements.

Distribution of mineral elements within the trees

As to the mineral composition of various parts of the trees of each group in the experiment, it appears (table X) that elemental starvation in these fruit trees caused considerable redistribution of mineral elements within the various organs of the tree.

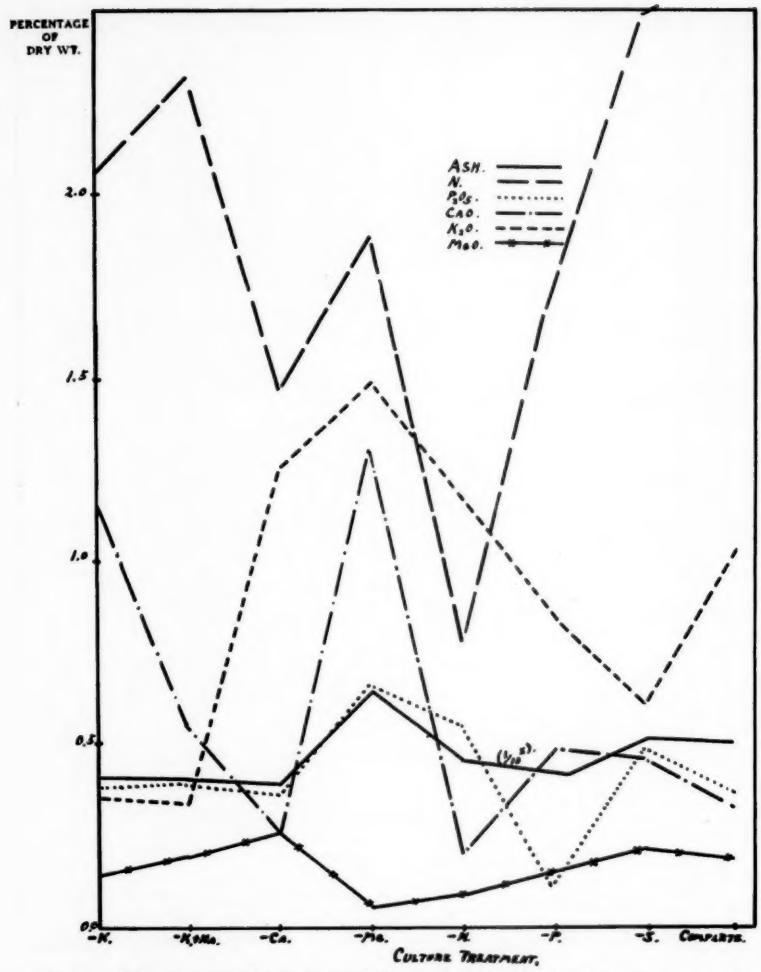

FIG. 9. Mineral composition in percentage of dry weight of branch (one and two year) roots from French prune trees starved for given elements.

Minus-potassium group.—The upper parts of these trees were high in N as compared with normal (or complete-solution) trees. The leaves (all leaf tissue referred to in this section was harvested July 25, 1930) especially were high in both N and P. The lower parts of the trees were low in N; the wood was high in P, and Fe_2O_3 was two to three times more than normal. Young roots were high in calcium and low in Mg. The parts of the trees

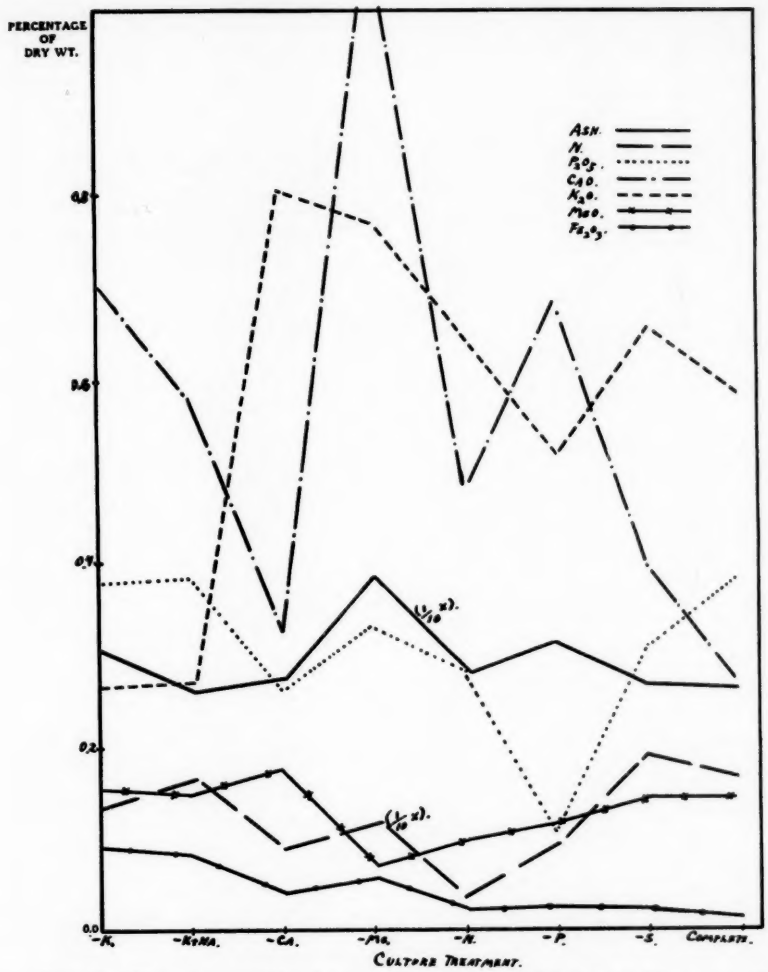

FIG. 10. Mineral composition in percentage of dry weight of main (three-year-old) root tissues of French prune trees starved for given elements.

lowest in K were: roots, about 41 per cent. normal in K; and trunk wood, 40 per cent. normal.

Minus-potassium plus-sodium group.—The composition was very much as in the preceding, but the entire trees were high in P. Perhaps this great absorption of P is characteristic of trees treated liberally with sodium salts.

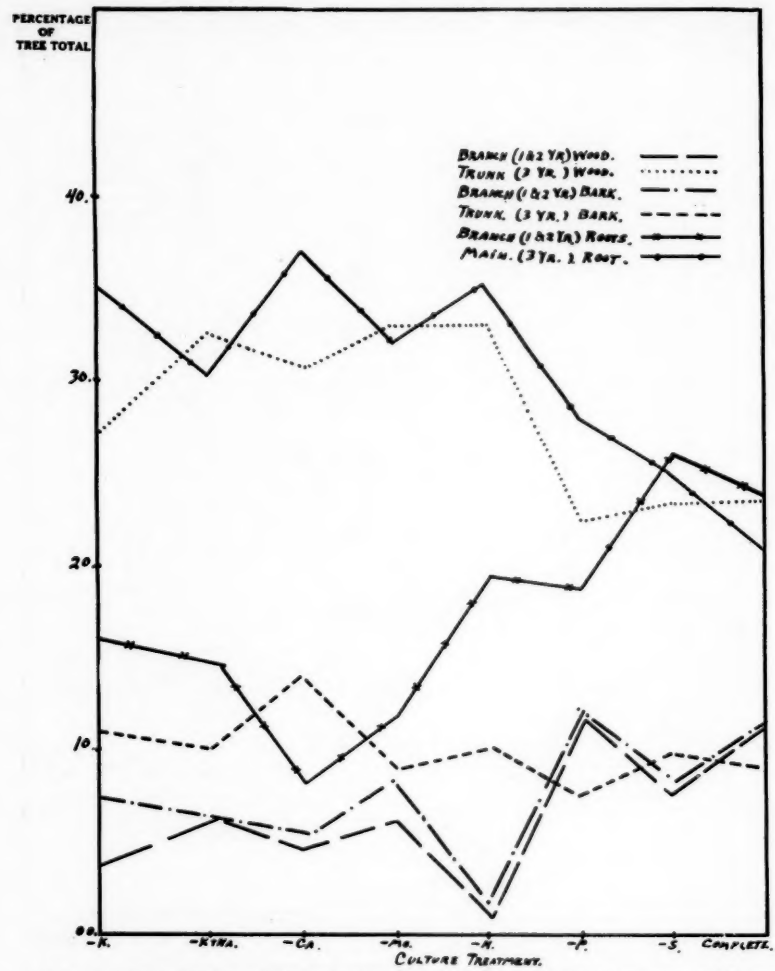

FIG. 11. Distribution of dry weight among the various parts of two-year-old French prune trees at close of the starvation experiment.

Minus-calcium group.—The entire trees were low in N. Leaves showed only 35 per cent. of normal Ca content. Wood and main roots had abundant Ca, apparently immobile. Young bark showed a Ca content which was 21 per cent. of normal, apparently being the most severely Ca-starved tissue of the tree. Trunk wood was low in Fe.

Minus-magnesium group.—The leaves were high in N, the lower part of the tree being low in N; leaves were low in Ca, the rest of the tree being

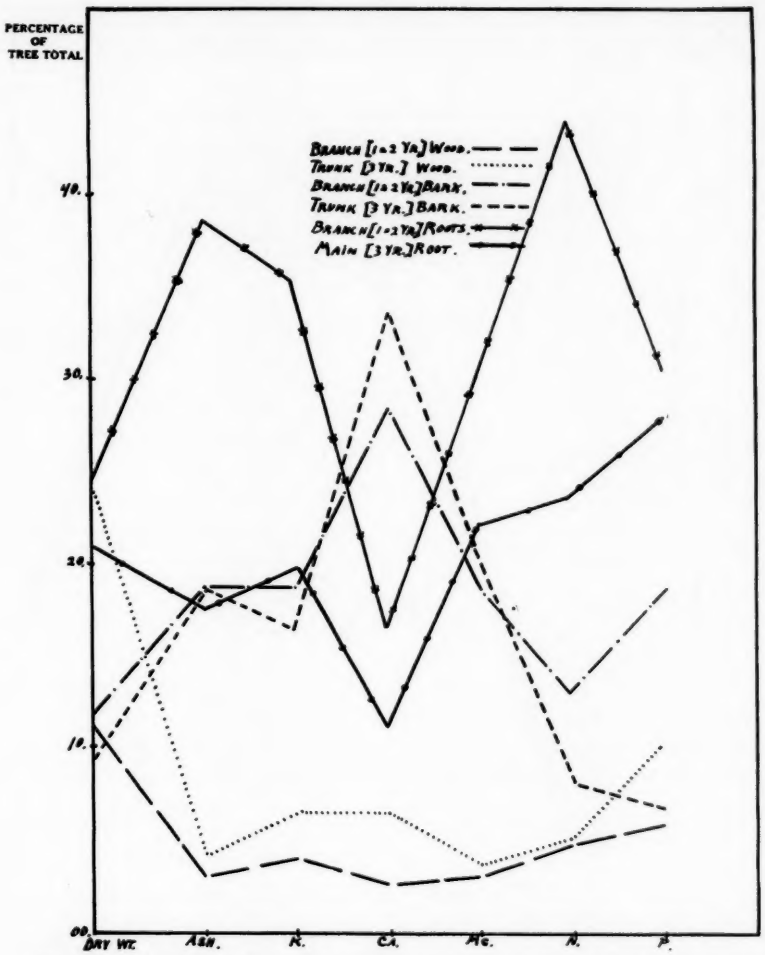

FIG. 12. Distribution of mineral elements among the various parts of French prune trees grown for two years in complete-culture solution; given in percentage of total weight of the given element in the tree.

high in that element. The leaves and young roots were high in P, but the rest of the tree was low in P. The wood was high in Fe. The entire tree was high in ash. The tissue lowest in Mg, in percentage of normal, was leaf tissue, with Mg 19 per cent. of normal.

Minus-nitrogen group.—The upper parts of the trees were high in Mg; the wood and young roots were high in K; wood and main roots were high

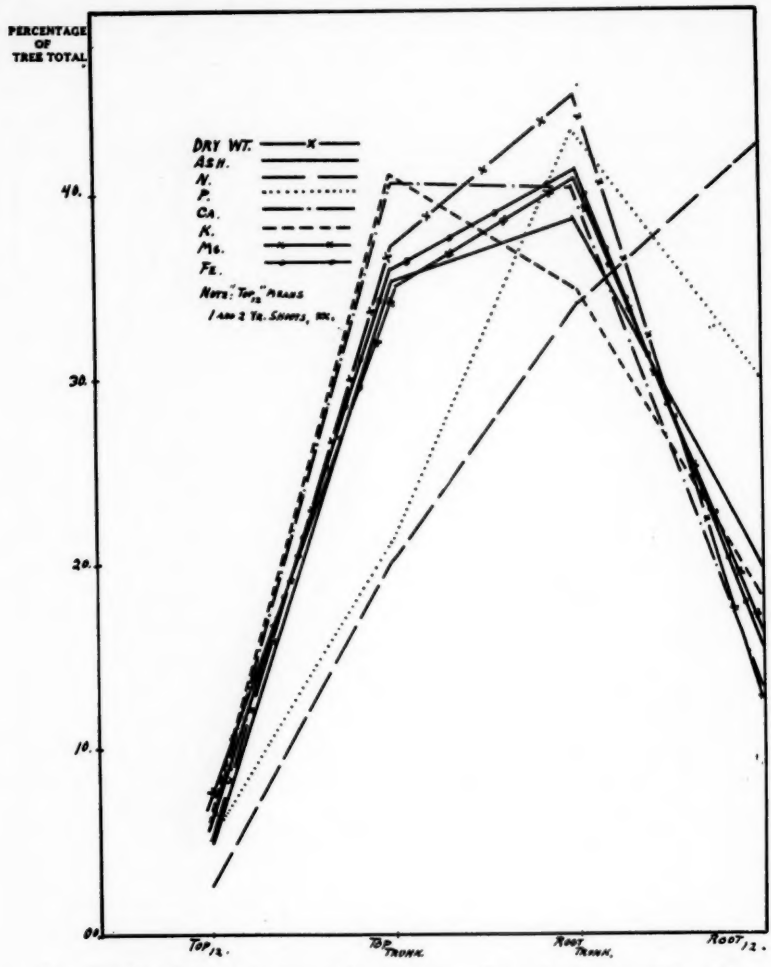

FIG. 13. Distribution of mineral elements among the various parts of French prune trees after two years of growth in distilled water.

in Ca; young roots and bark were low in Ca. The main root showed most severe N starvation, with a N content only 17 per cent. normal.

Minus-phosphorus group.—The leaves were high in N, but the remainder of the tree was low in this element. The upper parts of the tree showed high Mg, but the rest of the tree was low in it. The trunk parts were high

in Ca. The main root portion was severely starved for P, with only 26 per cent. normal content; trunk bark had 27 per cent. normal P, and leaf tissue 28 per cent.

Minus-sulphur group.—The young wood and leaves (July 25) were rather low in N, but the rest of the tree was very high in this element. The entire tree, except the young roots, was low in P. The upper parts of the trees were high in ash. Ca was high throughout the entire tree. Leaf tissue contained only about 15 per cent. of the normal S content.

Distilled-water trees

French prune trees grown in distilled water for two years (tables IX–XIV) showed:

	Percentage of normal
Young branch (1 and 2 years) tops	N = 27 CaO = 70 K_2O = 84
Trunk top	N = 39 CaO = 18 K_2O = 97

Young roots showed N 55 per cent. of normal, K_2O 55 per cent of normal, ash 76 per cent. of normal, MgO and CaO equal to that of complete-solution trees. Main roots had 19 per cent. normal N, 86 per cent. normal ash, low MgO, high CaO, and 50 per cent. normal P_2O_5.

It should be noted that the N content of the main root of these trees fell to the same level as in the main root of –N trees; but the young roots of the latter showed far more drastic N starvation than did young roots of distilled-water trees.

As to the complete-solution trees, it is to be noted that 45 per cent. of the dry weight of the tree was in the root at the close of the experiment (see fig. 11). With the exception of CaO, the bulk of the mineral total was in the root (leaves not being included). As for CaO, 63.2 per cent. of the tree total was in the bark of the top.

Root-top distribution of mineral elements

Roots of complete-solution trees contained:

68.8	per	cent.	of	total	N	of	the	tree
58.5	"	"	"	"	P_2O_5	"	"	"
55.9	"	"	"	"	Ash	"	"	"
27.4	"	"	"	"	CaO	"	"	"
53.9	"	"	"	"	MgO	"	"	"
55.2	"	"	"	"	K_2O	"	"	"

TABLE XI

DISTILLED WATER TREES

TOTAL MINERAL CONTENT (GM. PER TREE) AND DISTRIBUTION AS SAME

AVERAGE MINERAL CONTENT (ENTIRE TREE) AS PERCENTAGE OF DRY WEIGHT

DIVISION	GROSS DRY WEIGHT	TOTAL	GROSS ASH	TOTAL	GROSS N	TOTAL	GROSS Fe_2O_3	TOTAL
	gm.	%	*gm.*	%	*gm.*	%	*gm.*	%
Top 1, 2	4.0	4.2	0.124	4.9	0.0108	2.5	0.0012	6.3
Top trunk	36.4	37.3	0.910	36.5	0.0873	20.5	0.0069	36.5
Root 1, 2	13.0	13.4	0.494	19.8	0.1820	42.8	0.0030	15.4
Root trunk	44.0	45.1	0.968	38.8	0.1450	34.1	0.0079	41.8
Total	97.0	100.0	2.496	100.0	0.4251	100.0	0.0190	100.0
Tree average	.....	.....	2.57%	.....	0.438%	.....	0.0195	.....

	CaO		MgO		K_2O		P_2O_5	
Top 1, 2	0.0190	5.6	0.0094	6.6	0.0199	5.5	0.010	5.1
Top trunk	0.1383	40.9	0.0508	35.7	0.1490	41.2	0.042	21.5
Root 1, 2	0.0442	13.1	0.0231	16.2	0.0656	18.1	0.058	30.0
Root trunk	.1360	40.3	.0589	41.5	.1265	35.2	0.085	43.4
Total	0.3375	100.0	0.1422	100.0	0.3610	100.0	0.195	100.0
Tree average	0.348%	.....	0.146%	.....	0.372%	.....	0.201%	.....

Roots of distilled-water trees contained:

76.9 per cent. of N of the tree
73.4 " " " P_2O_5 " " "
58.6 " " " Ash " " "
53.4 " " " CaO " " "
57.7 " " " MgO " " "
53.3 " " " K_2O " " "

The iron content of the young wood (and of main roots) of $-K$, $-K+Na$, $-Mg$, and $-S$ trees was excessively great, in proportion to the amount of Fe present in young bark tissues of these same trees. Iron appeared to have difficulty in reaching the bark under these conditions of starvation. On the other hand, young bark tissues of $-P$ and $-Ca$ trees were excessively high in iron, while the iron content of young wood and main root sections of these trees was comparatively very low. The work of INGALLS and SHIVE (**4**) on iron in lime-loving annual plants tends to confirm this point.

It should be stated here again that the $-K$, $-K+Na$, $-Mg$, and $-S$ trees were all fed larger amounts of iron than were the complete, $-Ca$, or

– P trees. If all groups had been given equal amounts of iron, probably its total absorption by – K, – Mg, or – S trees would have been well below that by complete-solution trees.

All tissues analyzed for iron were washed in weak HCl at the time of harvesting the trees. In spite of this treatment, the small roots apparently retained large amounts of iron as impurity on the surface of the rootlets. The other tissues of the trees were washed very nearly free of iron contamination.

About May 15 of the second year of starvation, pH readings were made on freshly expressed sap from nearly mature leaves of the trees of each of the experimental groups. Sap was expressed almost immediately after the leaves were picked. The results are as follows (reading by colorimetric method):

Leaves from trees grown in	pH
complete solution	4.7
– K "	5.3
– K + Na "	5.0
– Ca "	4.6
– Mg "	5.2
– N "	4.8
– P "	5.1
– S "	5.0

Elemental starvation was more effective in altering the pH of leaf sap than were changes in the pH of the culture solution itself. (Trees growing in solutions at pH 4 to pH 10 had leaves giving expressed sap of nearly identical pH.) At the same time (May 15) all leaves showed absence of nitrate ion, in appreciable quantity (by di-phenylamine test), except that low magnesium leaves, which though entirely green, showed at least 10–20 p.p.m. of nitrate in fresh tissue.

As to the general problems of fruit-tree nutrition and long continued growth of trees in water-culture medium, we still have much to learn before we can duplicate normal tree growth in fertile soil out-of-doors. Certainly aerated solutions are essential for fruit-tree culture work. LIVINGSTON has shown their desirability even for cultures of the comparatively smaller rooted annual plants. Also for maximum fruit-tree growth in water cultures an AZ type of solution will be necessary. HAAS (3) has recently reported that boron particularly is essential for normal growth of citrus seedlings. He described the symptoms produced on such trees by the absence of boron; for example, splitting and corking of the main veins of the leaves, together with other effects.

Much work has been done in the past in starving annual plants for one element at a time. A very interesting and little-known field lies open, in the study of starvation for two elements at a time, or the study of balance

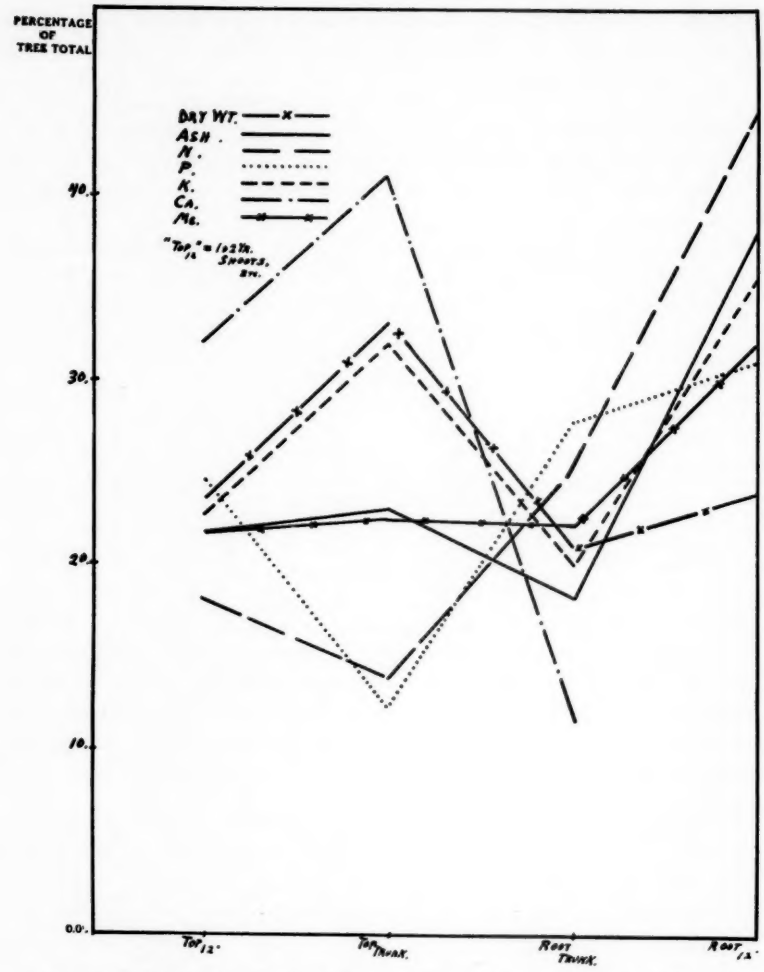

FIG. 14. Distribution of mineral elements among the various parts of French prune trees after two years of growth in complete solution (for comparison with figure 13).

between various ions. Already work has shown that a low-potassium plant also requires a low nitrogen supply in order to develop to full maturity and fruiting. In the writer's own experience, wheat plants low in both iron and sulphur developed to full maturity very quickly, heading out in normal fashion, but on short straw, even though the plants were always partially chlorotic. Plants low in both magnesium and iron offer interesting cases

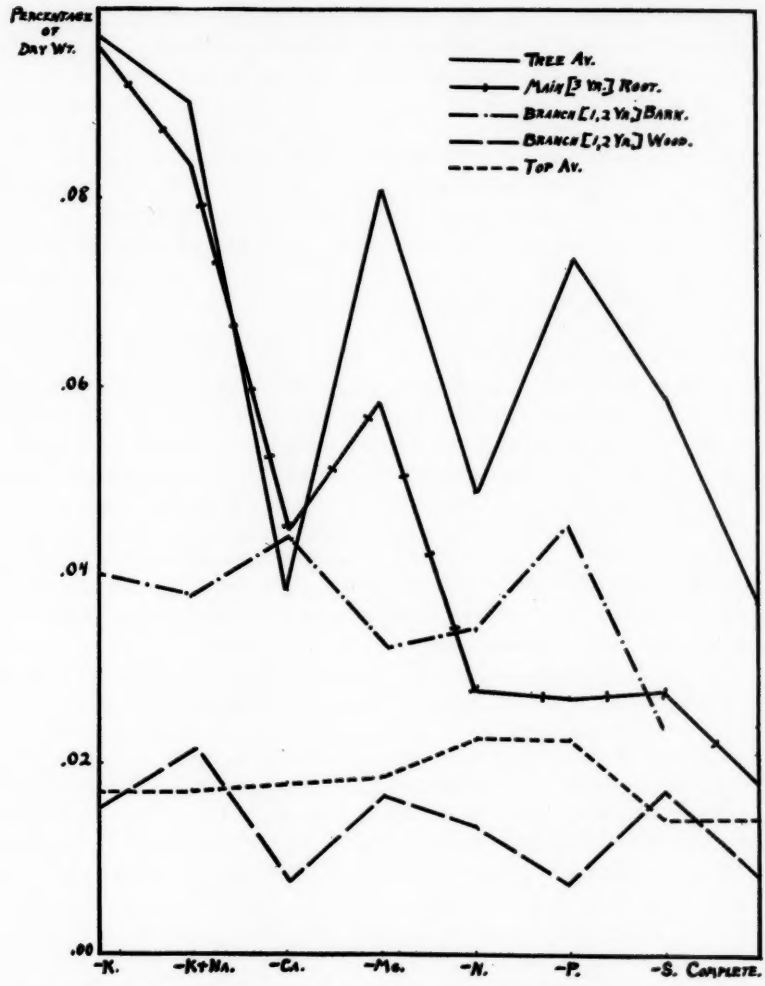

FIG. 15. Fe_2O_3 content of various tissues of two-year-old French prune trees starved for given elements.

for study. The phosphorus-iron ratio in relation to the development of "bronzing" could well be studied.

It may be asked here, are we justified in studying such problems as the nutrition, elemental starvation, or seasonal absorption of fruit trees, as grown in water cultures in the greenhouse, as a means of throwing light on

the physiology of trees as they grow in the orchard? What factors appear to govern seasonal nutrient absorption by perennial plants? The salt absorption by tree roots early in summer is apparently governed by the amounts of storage, both organic and inorganic, in the tree; by the supply of soluble salts in the soil; by the pH of the soil solution, etc.; by temperature or light intensity; and by the rate of growth of both tops and roots. The very early growth of the roots (while the top is still dormant) probably explains the marked absorption of calcium early in the spring, when other elements are often actually lost from the roots, or at least are not absorbed to any extent.

Later in the season, when the storage supplies within the tree are depleted, the rate and character of absorption depend less on storage material but more directly on recent photosynthesis, shoot growth, leaf area, and therefore on light and temperature levels. Still later in summer, after terminal shoot growth is over and only diameter increase is proceeding, salt absorption is less rapid, and is perhaps not so greatly influenced by recent photosynthesis but more by stored food. It may be noted that much of the salt absorbed late in summer and in fall is calcium, with some sulphate (and in trees in soil, large amounts of silica and chlorides). These materials largely go to fill up the leaves, prior to their abscission, and are lost from the tree with the fall of the leaves. Consequently the excessive late-season absorption of calcium, sulphate, etc., has little importance in the metabolism of the woody part of the tree that goes into the winter.

Certainly the various species of fruit trees do not all make their shoot growth at exactly the same period of the summer, and salt absorption seems to vary in a similar manner. Young apple trees, especially in the coastal districts of California, start growth rather late in the spring, continue terminal shoot growth late into the fall, and hold part of their foliage until December, in some years at least. Such trees appear to show a far greater late-season absorption of nitrate than do the earlier maturing French prune trees, etc., in the same climate. BARKER (1) in England reports that most of the root growth of apple trees occurs after top growth is over.

Summary

1. Starvation symptoms typical for each of the lacking elements were produced during the first year of starvation in French prune trees. Foliage symptoms were usually most strikingly characteristic and developed first, except in the case of calcium starvation, where root trouble is entirely dominant. Only with difficulty did such trees grow enough to show the leaf mottling characteristic of most low-calcium plants. Elemental starvation often produced changes in the mineral composition of leaf or bark tissues very different from those produced in wood, root, etc.

2. *Mg starvation* resulted not only in a low percentage of Mg in the leaves, but a Ca content actually lower than that found in – Ca leaves. Lack of Mg also resulted in high N and P in the leaves, very low N and P in the trunk wood, and very high ash content throughout the tree, except in leaf tissue.

3. *Ca starvation* resulted in both low N and low ash throughout the tree, except in the wood. Ca stored in the original trunk wood at the beginning of the experiment apparently remained there. The main root portion at the close of the experiment still showed abundant calcium, although leaf tissue had shown only 35 per cent. of normal calcium content.

4. *K starvation* resulted in leaf tissue high in N, P, Ca, Mg, and low in ash. Entire trunk parts and roots were low in N.

5. *N starvation* resulted in a low ash content except in the wood and very low N throughout. The young wood was peculiarly high in Ca and Mg, and normal in K and P. Young roots were low in Ca and Mg, and high in K. Main roots were extremely low in N (equal to 17 per cent. of normal), low in Mg and P, and high in Ca.

6. *P starvation* resulted in leaf tissue high in ash and N, extremely high in Mg and Fe, low in Ca, and P_2O_5 equal to 28 per cent. of normal. All the other parts were decidedly low in N. The roots and trunk were low in Mg.

7. *S starvation* resulted in leaf tissue low in N and in ash, and a SO_3 content equal to 15 per cent. of normal. The young wood was also low in N, but the rest of the tree was high in N, the average N content of the tree being 1.65 per cent. (dry weight basis), the highest of any group. Sulphur starvation decreased potassium absorption and increased absorption of Ca and Mg.

8. Trees grown in *distilled water* for two years had, at the conclusion of the experiment, a tree average of 30 per cent. normal N (on percentage of dry weight); 60 per cent. normal K_2O; 71 per cent. normal CaO; 70 per cent. normal P_2O_5; and 54 per cent. normal Fe_2O_3. The final dry weight was 28 per cent. that of complete-solution trees of the same age.

9. As regards iron distribution in French prune trees grown in water culture, excessive amounts of iron occurred in young wood and in root portions of – K, – K + Na, – Mg, and – S trees. The iron content of the bark was excessive only in cases of P and Ca starvation, in which cases the iron in the young wood and main roots was proportionately very low.

10. It appeared possible that potassium starvation for one year might be more detrimental to subsequent growth and absorption than a like period of total starvation.

11. Trees placed in distilled water finally made far better growth of top and root than did like trees placed in a nutrient solution containing all the usual elements except calcium. Trees placed in a simple solution of calcium

hydrate at pH 7.2 made far better growth than did trees in a minus-calcium solution.

12. Trees grown for two years in minus-potassium solutions, and transferred April 12 of the third year to a plus-potassium solution, responded quickly and developed excellent shoot growth and fine leaf color in about two weeks. Only a trace of the former chlorosis remained near the midrib portions of the leaf blade. Leaf color was obviously improved five days after the potassium was added to the culture solution. Later in summer, however, the tip leaves of the young shoots of these trees showed excessive reddening, then browning or burning, and drying up. The shoot tip itself died back 6 to 8 inches.

13. Other trees, also previously starved for potassium for two years, and placed in complete solution cultures on May 15, or on June 25 of the third year, showed no shoot growth response whatever to the added potassium. Leaf color did change in about 10 to 12 days, however, and the non-scorched areas of badly chlorotic, scorched leaves turned green except for small areas bordering on the scorched margins of the leaf blade. The roots of these trees all responded vigorously to added potassium, quickly making abundant root growth. Apparently it is impossible after about May 1 to stimulate shoot growth in these trees by adding potassium.

14. The pH of the expressed sap of the leaves of the starvation series showed that elemental starvation is more effective in altering pH of leaf sap than are changes in the pH of the culture solution itself. (Trees growing in solution at pH 10 and pH 4 had leaves giving expressed sap of nearly identical pH.) Freshly expressed sap taken from nearly mature leaves of trees starved for K, S, or Mg showed higher pH readings than did sap from complete-solution leaves.

15. At the same time (May 15) all leaves showed absence of nitrate in appreciable quantity, except the low-magnesium leaves, which though entirely green, showed at least 10–20 p.p.m. of nitrate in fresh tissue.

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DATE OF FLOWERING AS AFFECTED BY CLIMATIC TEMPERATURE

J. F. A. MACLAGAN

(WITH EIGHT FIGURES)

Introduction

The investigation reported here is mainly concerned with the effect of temperature on the flowering date of a number of genera of woody shrubs. Since the reproductive phase of a plant is not independent of its vegetative phase, and since temperature does not act independently of other climatic factors, before considering the special aspect of the problem,—the time of onset of the reproductive phase as influenced by temperature,—it seems advisable to examine briefly the wider question of how the plant as a whole is affected by climatic conditions.

INFLUENCE OF CLIMATIC FACTORS.—The effect on the plant of climatic conditions is not clear-cut. One climatic factor rarely if ever exercises an effect independently of other external factors, climatic, soil, etc. The reaction of the plant itself is complex. Climatic factors operate by their influence on the metabolism of the plant, and the reactions of the various metabolic functions are not uniform. These interacting units of the environment and the plant's metabolism affect flowering both as to quantity and time.

The primary effects of these climatic variations express themselves in a broader sense in the relationship which exists between the vegetative and reproductive phases of the plant. In this broader sense any factor which tends to stimulate the development and therefore the continuance of the vegetative phase must retard the onset of the reproductive phase. The date of first flowering may be regarded as the first marked outward indication of the onset of the reproductive phase. If this be accepted, then from its variation from year to year, any correlation which can be established between weather and such variation may be taken as evidence of the effect of climatic factors on the relationship between the two phases. Actually the formation of the first definite flower initial marks the initiation of the change from vegetative to reproductive phase. This being so, date of flowering is in reality a late indication of this change of phase. No data are available on this fundamental point regarding the time when change of phase commences. Following initiation of change of phase are many other stages in the march of events toward actual flowering. Most significant of these steps is the initiation and formation of the actual gametes. It will be indicated later that it is at the moment of gamete formation that temperature exerts its maximum effect.

Previous work has been concerned mainly with amount of flowering and fruit setting in each year, rather than with date of flowering. While the quantitative side of flowering is not necessarily affected in the same way as time, nevertheless it is related.

BARKER and LEES (**2**) dealt with the effect of weather on fruit bud formation at Long Ashton. What is called good "ripening" or "maturing" of the wood occurs when vegetative growth ceases somewhat early in autumn, thus favoring the formation of flower buds (change of phase) before winter sets in. The comparatively wet July and August at Long Ashton caused active vegetative growth to continue late into the autumn so long as the temperature was high enough to favor it, and fruit bud formation suffered in consequence.

LEES (**4**) in a later paper says: "When the influence of pests, diseases, manuring, pruning and stock influence are eliminated, the remaining factors of previous crop and summer rainfall determine the future crop in the large majority of cases."

INFLUENCE OF TEMPERATURE.—It must be clearly realized that temperature, although dealt with here almost as an isolated factor for the purposes of this investigation, cannot strictly be separated from the other components of the environment. Similarly, time of flowering is influenced not only directly by the weather but also indirectly by the other processes taking place in the plant. Hence a perfect correlation of temperature with time of flower opening is not to be expected, although, as will be shown later, temperature is of major importance.

Previous workers have used the summation of all temperatures above an arbitrary datum line over a certain period to express the total amount of effective heat incident, and so to determine the dependence of plant development on this factor (for example, LIVINGSTON **5**, and PALLADIN **6**). The results arrived at by such a method are unsatisfactory. Temperature above a certain point may be non-effective or deleterious; the usually depressant or positively injurious effect of frost is ignored; and no allowance is made for the physiological effects of change in temperature at different temperature levels. Simple change of temperature within short periods has been shown to have a profound effect on plant development, at least at germination.

FLOWERING DATE AS INFLUENCED BY TEMPERATURE.—In the present investigation the weather records and flowering records have been compared with a view to finding some period or periods of the year when weather has a marked and consistent correlation with date of flower opening. Temperature has been found to exhibit such a correlation. The mechanism through which the effect of temperature at particular times operates has been sought

principally in the condition of the different parts of the flower bud, particularly the sporophylls, at these times. In this regard it is of interest to note that BALL (**1**), quoting GOFF as his authority, says in connection with the development of the flower buds of the different fruit trees: "The order of development is the same in the different fruits, *viz.*, first the calyx appears, then the petals, stamens, and finally the pistil. All these parts are present before the winter sets in, the ovules and pollen grains not being formed until February or March of the following year. There are exceptions to this since GOFF found that in the gooseberry ovules were generally present before the winter set in." BALL found that in the buds of three varieties of plum, Victoria, Monarch, and Pond's seedling, all the flower parts were present in November but ovules and pollen grains were not usually formed until January or February.

WHYTE (**7**) makes an illuminating observation in connection with the differentiation of floral parts. "The subject of time of reduction does not appear to have received much attention in the past, but from observations made by the writer on several unrelated genera of flowering plants, it may be said that in all those examined a considerable interval always occurs between the reduction processes in the pollen mother cells and the megaspore mother cells in any given flower. Growth of the female tissues does not generally commence until complete tetrads are formed, and pollen has been developed for some time before reduction in the ovules. The interval between the reduction processes is probably governed largely by the amount of ovular development in the plant concerned."

COULTER and CHAMBERLAIN (**3**) mention the time of formation of the microsporangia and megasporangia for species in which the details are known. In the case of the microsporangia, different species exhibit considerable variation. They suggest that for those plants whose flowers open early in the season the mother-cell stage is the usual winter condition. At any rate the interval of time between formation of the archesporium and pollination is probably in many cases considerably longer than is generally supposed.

In the winter bud of *Quercus velutina* the stamens are well formed but the tissue is still homogeneous. The microsporangia of *Salix glaucophylla, Populus monilifera,* and *Symplocarpus,* however, pass the winter in the mother-cell stage. A further advance is seen in *Alnus glutinosa* and *Corylus americana,* whose mid-winter catkins contain pollen ready for shedding, with the generative cell formed.

The length of time taken for the development of megasporangia is probably also very variable and related to the seasonal habit of the plant. CHAMBERLAIN has found that the megaspore mother cell of *Salix* and *Populus* is not formed until the winter dormancy of the plant is over

and growth renewed in spring. In *Erythronium* the mother-cell stage is reached by the end of November and persists until early spring. The mother-cell stage has been found in *Acer rubrum* in March, indicating that in this species also the mother cell is the condition in which the winter is passed. It is to be noted that in *Salix* and *Populus* the microsporangia pass the winter in the mother-cell stage, but the megaspore mother cell is not formed until spring, a considerable interval of time elapsing between the processes of formation in the two classes of spore, male and female.

Records of data

The records of flowering dates on which this work is based are those kept at the Royal Botanic Garden, Edinburgh (unpublished). The records consist of lists of those plants which came into flower during each week of the year. Occasionally the entries have been made at fortnightly intervals instead of at the end of each week, and sometimes the period has been extended to three weeks, or even a month. Such extensions are most frequent in the case of early spring and late autumn records. The continuity of the records has been broken by the war and other causes, but sufficiently complete records are available for the years 1919 to 1929, excluding 1924. The temperature records used here are those taken at Edinburgh University and published in the monthly weather reports of the Meteorological Office, in conjunction with the daily weather records kept at the Royal Botanic Garden, Edinburgh (unpublished).

The data have been organized into graph form, and in each case the graph of the yearly flowering date has been plotted in juxtaposition to the graph of the significant weather belt or belts.[1] The coordinates of the temperature graph are: abscissae, years; and verticals, average temperature of significant belt. In the graphs of the date of flowering the abscissae are years, and the verticals are the deviation in each year from the average date of flowering over the period in question. The graphs are supplemented by tables giving in greater detail the data from which the graphs have been drawn.

Consideration of weather data more detailed than monthly averages has not been attempted except in a very few instances. Not only the action of other external factors, but also the relative lack of accuracy of the flowering records would tend to invalidate any attempt at a finer correlation if a more detailed analysis were carried out.

As might be expected from a general knowledge of the annual periodicity of plants, correlations between weather and flowering date appear

[1] "Weather belt" is defined as the weather obtaining over a period of time, from one to three months, which by analysis has been found to be significant in causing, by its deviation from normal, a deviation from the normal flowering time of each species.

most clearly in autumn and spring. As pointed out, many species form the flower buds in the autumn of the year previous to their opening. In such species "wood maturation" will have an important effect on the subsequent year's flowering. Rainfall is of importance as well as temperature, since drought causes the cessation of vegetative growth and thus allows the reproductive phase to commence.

As different stages of development are attained in the flower buds of different species before winter sets in, and the final stages are accomplished when activity recommences in spring, the autumn weather probably affects different species to different extents. An almost complete theoretical graded series can be erected in connection with degree of development of the reproductive phase in different species in winter.

At one end of the series are species which over-winter with the buds in the purely vegetative condition, no attempt at differentiation being made. Perennials of this nature must be only a single stage more complicated in their weather response than annuals. While the weather prior to winter may affect their physiological state and therefore presumably their subsequent flowering, it will be the weather of the spring (and summer) immediately prior to flowering which will be significant.

The next stage in the series is that of perennials which proceed to differentiation of buds prior to the onset of winter, but which cease differentiation at some stage before reduction and spore formation. Such plants over-winter with two classes of buds, leaf buds and incipient flower buds. The weather prior to over-wintering must affect such differentiation and therefore flower production in the following year. How far this is significant cannot be stated. Gardeners believe that a plant with formed flower buds can be, by appropriate manipulation of external factors in early spring, induced to change its "intentions," in so far that the flower buds do not produce flowers but return to vegetative growth. Such reversal might be possible in plants of the class falling into this grade, but it does not seem possible for plants of the next grade, which is that of perennials that over-winter with flower buds so differentiated that the sporophylls have assumed their definite character. A slightly more advanced stage is that when not only are the sporophylls definite in character, but the spores and gametes, at least on the microspore side, are definitely formed.

In perennials of the first class, autumn weather can have comparatively little effect on date of flowering in the following year. In those of succeeding classes, the weather of the autumn preceding any flowering year gains increasing importance as we ascend the series. In essence the statement may be made that, where the species proceeds in its development to a somewhat advanced stage of the reproductive phase before entering the winter dormant period, a significant weather belt must exist in the autumn

prior to the flowering year. Furthermore, as has already been indicated from the literature, and as will be shown in actual cases later, since the change of phase usually occurs in two steps (male development prior to over-wintering and development subsequent to over-wintering), two significant weather belts may exist. The weather belts coincide with the time of the respective steps in development. In the case of those plants which over-winter entirely in the vegetative phase, one major weather belt may be looked for in spring prior to flowering. Finally, the actual process of bud bursting and flower opening in all species must be conditioned to some extent by weather, and therefore another type of weather belt may be expected. The effect of frost on the opening buds is especially significant, more from the point of view of the records. The gardener observer, seeing frost-blasted buds, would probably not record the plant as having flowered, but would wait for a second crop of buds to open before entering the plant into the records. Three main significant weather belts may therefore be envisaged: (1) a belt occurring in autumn after one year's flowers have fallen, and far removed in time from the next flower opening, called here the *distal belt*; (2) another belt occurring in spring or early summer, nearer in time to date of flowering, called here the *proximal belt*; and (3) the weather just at or about flowering time, called here the *immediate belt*.

Soon after the work was commenced it was realized that, owing to the method of compiling the flowering records at intervals of a week or more, the experimental error in dealing with single species might be so large as to obscure the clear outlines. Consequently the genus was taken as the unit for comparison in order to establish the general rules governing its behavior if such existed. Only those species in each genus which were completely recorded were considered and incorporated. While it was felt that the members of a genus were related sufficiently closely to be thus dealt with as a unit, the possibility of single species showing individuality has not been lost sight of, and these are detailed when they occur.

While in general the results obtained point to temperature as a master factor in flower bud development, particular cases often cannot be explained on this basis. This is to be expected, however, in view of the fact that other factors come into play and may have a modifying, and sometimes an overriding, effect. For example, rainfall would affect the nitrate content of the soil and thus (or even by modified water supply) affect the interrelationship between the vegetative and reproductive phases. Internal factors may also have a potent, although less easily defined, influence. For example, it is generally accepted that a year of profuse flowering is followed in the subsequent year by a paucity of flowers, an effect which seems to hold despite favorable external conditions in the second year. The ques-

tion of characters of a genetical nature cannot be considered here, and in any case the clear lines of their possible effect are blurred by the nature of the records. Further, the question of imposed rhythm might be pertinent, but the fact that the records of most species used are of long standing militates against this.

Presentation of data

Rhododendron

It was realized at the commencement of the analysis that "maturation of the wood" in autumn is an important feature of this genus. A comparison of the autumn conditions, however, failed to show any correlation with time of flowering. On further study of the records it became clear that a weather belt occurs rather earlier in the year, about one to two months after flowering, which can be correlated with date of flowering in the following year. Unpublished data supplied by Miss F. B. Murray to the effect that the formation of pollen in the next year's flower buds may reach an advanced stage in development at a comparatively early date, within some two months after flower fall, strengthens this assumption, especially in view of the graded series already indicated. A striking case is seen in ***Rhododendron souliei,*** which in 1930 came into flower in the first week of June, and had flower buds with pollen formed by the first week of July.

As the species of ***Rhododendron*** considered here flower over a period of five months (February to June), the distal weather belt is not the same for all; hence the genus has been divided into three groups according to the time of flowering of the species. Each group consists of species which on the average flower practically together.

While the temperature at this distal weather belt showed a decided influence on time of flowering, it was found that in all species the correlation failed to hold in the years 1922 and 1923. This was accounted for when a proximal weather belt was taken into consideration. The average temperature of the subsequent January–March period was found to provide an explanation of these aberrations when it emerged as the proximal weather belt. Cytological evidence again supported the contention, for though the pollen may reach an advanced stage in the autumn, the development of the ovary does not take place until the spring. Indeed, reduction division may not take place until a very short time before flowering (unpublished data by Miss Murray).

Graphs are presented to illustrate this combined effect on time of flowering of the temperature at two different periods. In each group of species the most fully recorded species has been chosen to represent the group. In the graph of temperature for the distal belt, 1918 temperature has been

plotted on the same vertical as flowering for 1919, and so on, in order to facilitate direct comparison.

In addition to the graphs, tables are appended giving the complete data for all members of each group.

TABLE OF *RHODODENDRON* SPECIES

SPECIES	AVERAGE FLOWERING DATE
GROUP I:	
R. barbatum	February 28
GROUP II:	
R. metternichii	March 23
R. ciliatum	March 28
GROUP III:	
R. schlippenbachii	April 29
R. vaseyi	May 4
R. roylei	May 10
R. decorum	May 11
R. smirnowii	May 20

GROUP I: SPECIES FLOWERING IN FEBRUARY

Although only one species falls into this group, its agreement with the general behavior of the genus seems to justify its inclusion.

The distal temperature belt is April/May, the proximal temperature belt January/February. Figure 1 and table I refer to this group.

Comparing first the graphs of flowering time (fig. R1c) and April–May temperature of the preceding year (fig. R1a), it will be seen that, in general, high temperature corresponds to early and low temperature to late flowering, although this belt is separated from the actual flowering date by nearly nine months. In this species, however, as in most other species of the genus, in the years 1922 and 1923 the reverse was true, that is, flowering in 1922 was late and in 1923 early, while the temperature of the distal belt in 1921 was high and in 1922 low. Again in the year 1919 flowering was very late although temperature of the distal belt was average. Comparing the temperature graphs of the distal and proximal belts (fig. R1a and R1b), it will be seen that they agree except in those years of aberration 1919, 1922, and 1923. In other words, the proximal temperature had an overriding effect in these years. The extremely cold spring (proximal belt) of 1919 caused flowering to be late in that year, in spite of average warmth in the distal belt (April–May, 1918). The cold spring of 1922 had the same effect, and the comparatively warm spring (proximal belt) of 1923 made

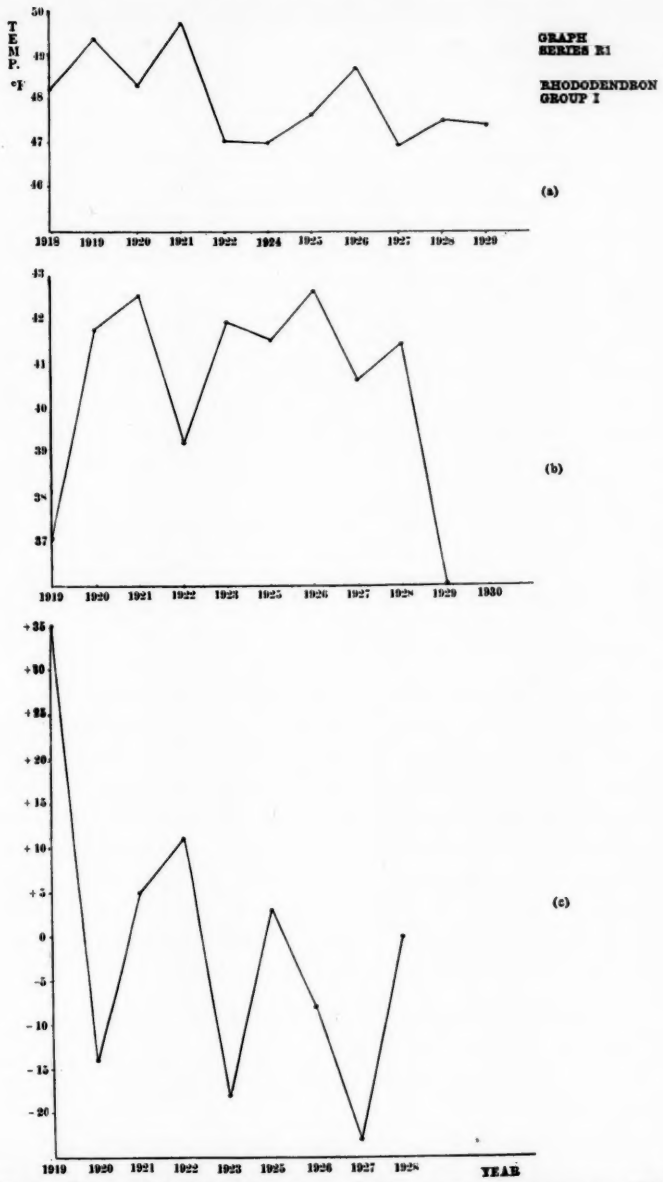

FIG. 1. Curves of distal temperatures (R1a), proximal temperatures (R1b), and deviations from average flowering time (R1c), of *Rhododendron barbatum*, 1918–1929.

flowering early in that year in spite of the less-than-average temperature of the previous April–May (distal belt).

TABLE I

Rhododendron, Group I

Year of flowering	Average temperature		*R. barbatum*	
	Distal (April–May)	Proximal (January–February)	Date of flowering	Deviation from average
1919	48.20	37.05	Apr. 5	+35.0
1920	49.35	41.75	Feb. 14	−14.0
1921	48.30	42.50	Mar. 5	+ 5.0
1922	49.70	39.20	Mar. 11	+11.0
1923	47.05	41.90	Feb. 10	−18.0
1925	47.00	41.50	Mar. 7*	+ 3.5
1926	47.65	42.60	Feb. 20	− 8.0
1927	48.70	40.60	Feb. 5	−23.0
1928	46.95	41.40	Mar. 3	+ 0.5
1929	47.50	36.00	.....	.....

* Record made at end of a fortnight.

Group II: Species flowering in March

For this group the distal temperature belt is June, the proximal temperature belt January/March. Figure 2 and table II refer to this group.

For comparison of flowering date and weather, *R. metternichii* has been adopted as an example and its graph drawn. If the graphs of figure R2a (distal belt), R2b (proximal belt), and R2c (flowering curve) are compared, it will appear that here as in group I the correlation of flowering with distal temperature (in this case June) holds except in the years 1919, 1922, and 1923; and as in the case of group I, this is clearly accounted for by the temperature which obtained in the proximal belt affecting these years.

Group III: Species flowering in April and May

The distal temperature belt affecting this group is June/July, and the proximal temperature belt is January/March. Figure 3 and table III refer to this group.

R. vaseyi has been chosen to exemplify this group, and graphed for purposes of comparison with the temperature belts. Figures R3a (distal tem-

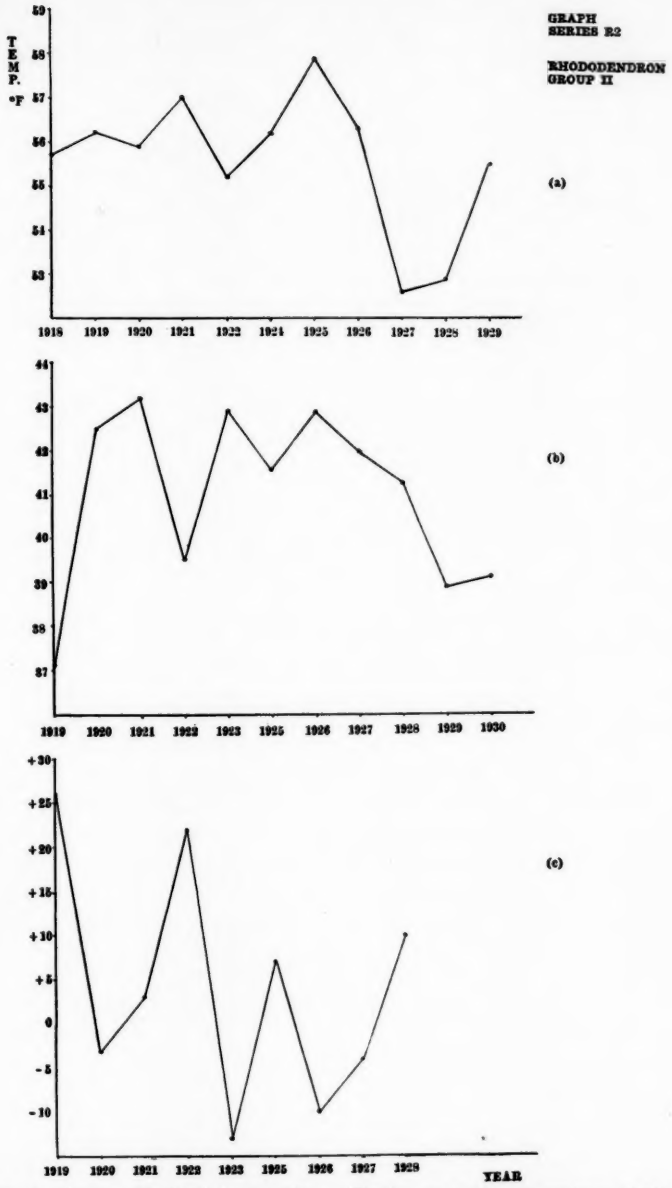

FIG. 2. Curves of distal temperatures (R2a), proximal temperatures (R2b), and deviation from average flowering time (R2c), of *Rhododendron metternichii*, 1918–1929.

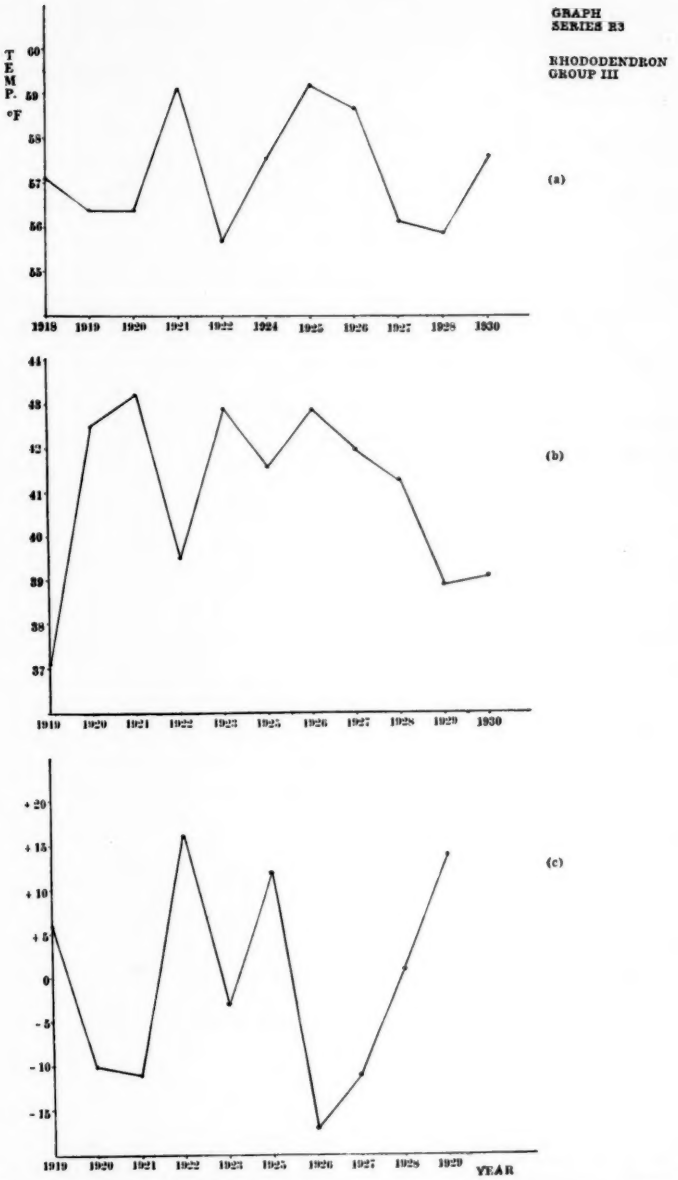

FIG. 3. Curves of distal temperatures (R3a), proximal temperatures (R3b), and deviation from average flowering time (R3c), of *Rhododendron vaseyi*, 1919–1929.

TABLE II

Rhododendron, Group II

Year of flowering	Average temperature		*R. metternichii*		*R. ciliatum*	
	Distal (June)	Proximal (January–March)	Date of flowering	Deviation from average	Date of flowering	Deviation from average
1919	55.7	37.1	Apr. 19	+26.0	Apr. 19	+21.0
1920	56.2	42.5	Mar. 6	− 3.0	Mar. 20	− 8.0
1921	55.9	43.2	Mar. 19	+ 3.0	Mar. 26	− 2.0
1922	57.0	39.5	Apr. 8	+22.0	Apr. 15	+17.0
1923	55.2	42.9	Mar. 10	−13.0	Mar. 10	−18.0
1925	56.2	41.6	Apr. 4*	+ 7.5	May 2†	.....
1926	57.9	42.9	Mar. 13	−10.0	Mar. 13	−15.0
1927	56.3	42.0	Mar. 19	− 4.0	Mar. 19	− 9.0
1928	52.6	41.3	Apr. 7*	+10.5	Apr. 7*	+ 5.5
1929	52.9	38.9	.....	.....	.....	.....

* Record made at end of a fortnight.

† Record made at end of three weeks.

perature), R3b (proximal temperature), and R3c (flowering date) show the same features as the graphs for groups I and II.

Cytisus

This genus provides a contrast to *Rhododendron,* for in the case of *Cytisus* no distal temperature belt affecting time of flowering could be discovered. An examination of the flower buds of a number of species was made toward the end of February and no pollen was found. If, as seems likely, the flower bud passes the winter with the stamens and ovary in an undifferentiated condition, it is not to be expected that the autumn weather will have any marked effect on time of flowering. Thus in *Cytisus* no distal weather belt will occur.

Table of *Cytisus* species

Species	Average flowering date
C. kewensis	April 20
C. beanii	April 21
C. ardoinii	April 21
C. biflorus	April 29
C. purgans	May 7

C. decumbens	May	8
C. hirsutus	May	12
C. albus	May	13
C. horniflorus	May	15
C. versicolor	May	17
C. glabrescens	May	21
C. purpureus incarnatus	May	22
C. scoparius	May	23
C. scoparius prostratus	May	23
C. scoparius andreanus	May	26
C. purpureus albus	May	28
C. sessifolius	May	29

Cytisus, ALL SPECIES

The data show the month of March as the period during which temperature exercises its particular influence. Figure 4 illustrates this point. Figure 4, C1a, shows March temperature, and C1b is a composite graph for seventeen species of *Cytisus*. This composite graph is compounded from the average of the figures representing the deviations from the average flowering date shown in each year by each of the seventeen species. The figures for each individual species are given in table IV, columns 3 to 36; and the figures derived from these, from which the composite graph has been drawn, are shown in the last column of the same table. In finding the average for 1920, the figure for *C. horniflorus* has been ignored, since if it were included its enormous deviation (−70) would give the average a false value. The two curves *a* and *b* show a remarkably close correlation, except that in the years 1923, 1927, and 1929 flowering was rather late. These deviations will be explained later by a more detailed consideration. Following these is a graph (fig. 4, C1c) for a single species, *Cytisus beanii*, which shows a remarkably perfect correlation with the temperature curve. As will be seen in table IV, the other species behave in the same way with minor deviations.

Cytisus as a whole seems to be rather an unstable genus, or perhaps easily affected by external conditions not evaluated here. There are a number of aberrations occurring in single species for which an explanation cannot be attempted. Such aberrations are very marked in the year 1929, and are the cause of lack of correlation between the composite graph (fig. 4, C1b) and the March temperature graph (fig. 4, C1a) in that year.

While the data do not permit explanation of these minor departures from the rule, two major departures may be accounted for on the general thesis developed here. These two deviations are seen, one in 1923 and the

TABLE III

Rhododendron, Group III

A, date of flowering; B, deviation from average date of flowering

Year of flowering	Average temperature		*R. schlippenbachii*		*R. vaseyi*		*R. roylei*		*R. decorum*		*R. smirnowii*	
	Distal (June–July)	Proximal (January–March)	A	B	A	B	A	B	A	B	A	B
1919	57.1	37.1	May 17	+18.0	May 10	+6.0	May 17	+7.0	May 17	+6.0	May 24	+4.0
1920	56.4	42.5	Apr. 24	−5.0	Apr. 24	−10.0	Apr. 17	−23.0	May 1	−10.0	May 8	−12.0
1921	56.4	43.2	.....	.....	Apr. 23	−11.0	May 7	−3.0	May 7	−3.0	May 7	−13.0
1922	59.1	39.5	May 13	+14.0	May 20	+16.0	May 20	+10.0	May 20	+9.0	May 27	+7.0
1923	55.7	42.9	May 5*	+2.5	May 5*	−2.5	May 5*	−8.5	June 2	+22.0	May 19	−1.0
1925	57.55	41.6	.....	.....	May 16	+12.0	May 25	+15.0	May 2†	.....	May 25	+5.0
1926	59.2	42.9	Apr. 10	−19.0	Apr. 17	−17.0	Apr. 17	−23.0	May 1	−10.0	May 29	+9.0
1927	58.7	42.0	Apr. 16	−13.0	Apr. 23	−11.0	May 21	+11.0	Apr. 16	−25.0	May 7	−13.0
1928	56.16	41.3	.....	.....	May 5	+1.0	May 12	+2.0	May 19	+8.0	May 19	−1.0
1929	55.9	38.9	.....	.....	May 18	+14.0	May 18	+8.0	May 18	+7.0	June 1*	+8.5

* Record made at end of a fortnight.

† Record made at end of three weeks.

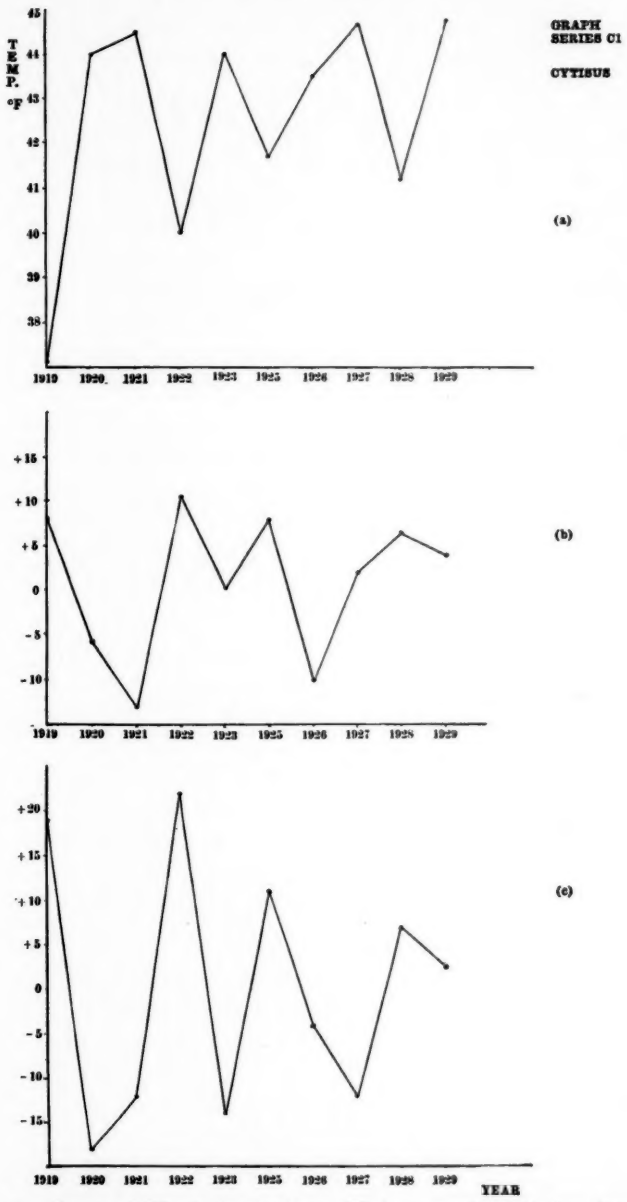

FIG. 4. Curves of March temperatures (C1a), composite deviation from average flowering time for 7 species of *Cytisus* (C1b), and deviation from average flowering time for *Cytisus beanii* (C1c), 1919–1929.

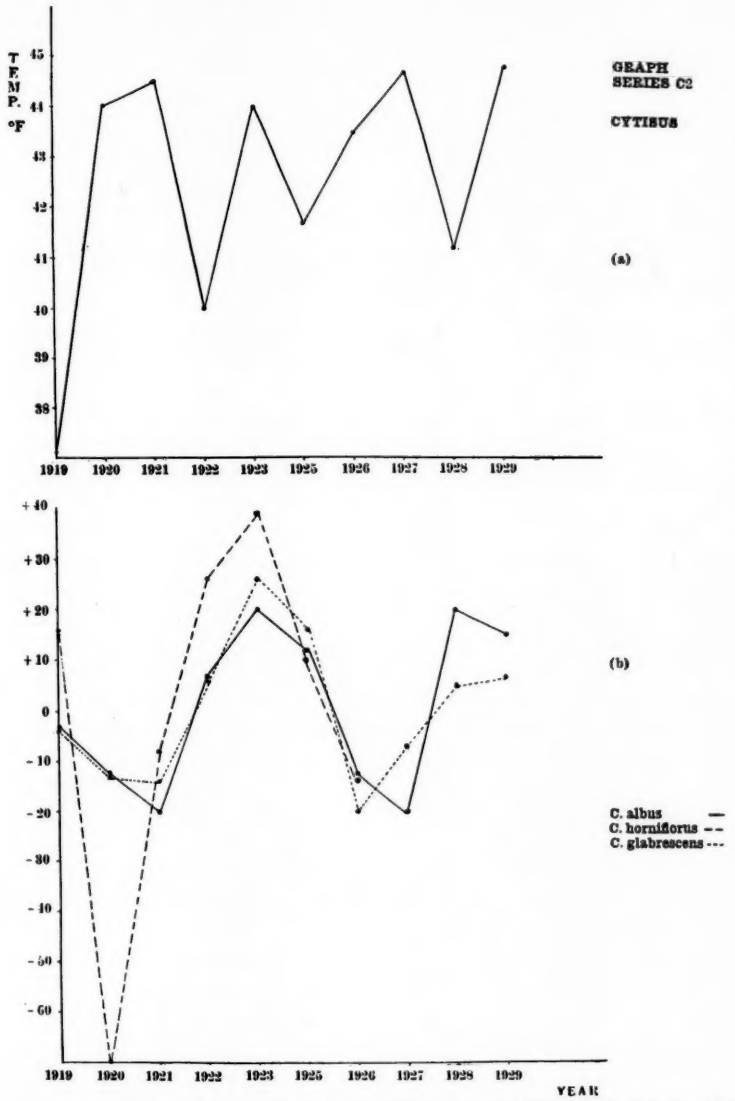

FIG. 5. Curves of March temperatures (C2a), and deviation from average flowering time for *Cytisus albus, C. horniflorus,* and *C. glabrescens* (C2b), 1919–1929.

other in 1927. In 1923 three species, *C. albus, C. horniflorus,* and *C. glabrescens,* differed from the others in flowering very late.

Species	Average flowering date	Flowering date in 1923
C. albus	May 13	June 2
C. horniflorus	May 15	June 23
C. glabrescens	May 21	June 16

This is illustrated in figure 5.

In 1927 other three species, *C. decumbens, C. versicolor,* and *C. scoparius,* flowered later than their fellows.

Species	Average flowering date	Flowering date in 1927
C. decumbens	May 8	May 28
C. versicolor	May 17	May 28
C. scoparius	May 23	June 4

This is illustrated in figure 6.

Those species which were retarded in 1923 are the reason for the lack of correlation exhibited by the composite graph of the genus in 1923, and similarly those species which were retarded in 1927 explain the lack of correlation of the composite graph in 1927. This is illustrated in table V, where alongside the composite figures for all seventeen species (column A) are seen two sets of composite figures for fourteen species (columns B and C), where in one case the three species which were late in 1923, and in the other case the three species which were late in 1927, have been omitted. It will be seen that these columns (B and C) show their greatest deviation from column A in 1923 and 1927 respectively. If the figure in column A is replaced in 1923 by the figure in column B, and in 1927 by the figure in column C, the disturbing influence of these retardations, caused, as will be seen later, by cold spells, will be eliminated, and the A figures will be seen to fluctuate in accordance with the March temperature in a much more consistent manner.

The retardations of these species are examples of the effect of cold weather just at time of flowering (immediate temperature belt). In 1923 a cold spell set in on May 9 and lasted until the end of the month. The average temperature for May in that year was 47.5° F., several degrees below the usual May temperature. In 1927, in the last week of April, there were severe ground frosts, sufficient to retard flowering. The grass minimum temperatures recorded at the Royal Botanic Garden, Edinburgh, for this period are:

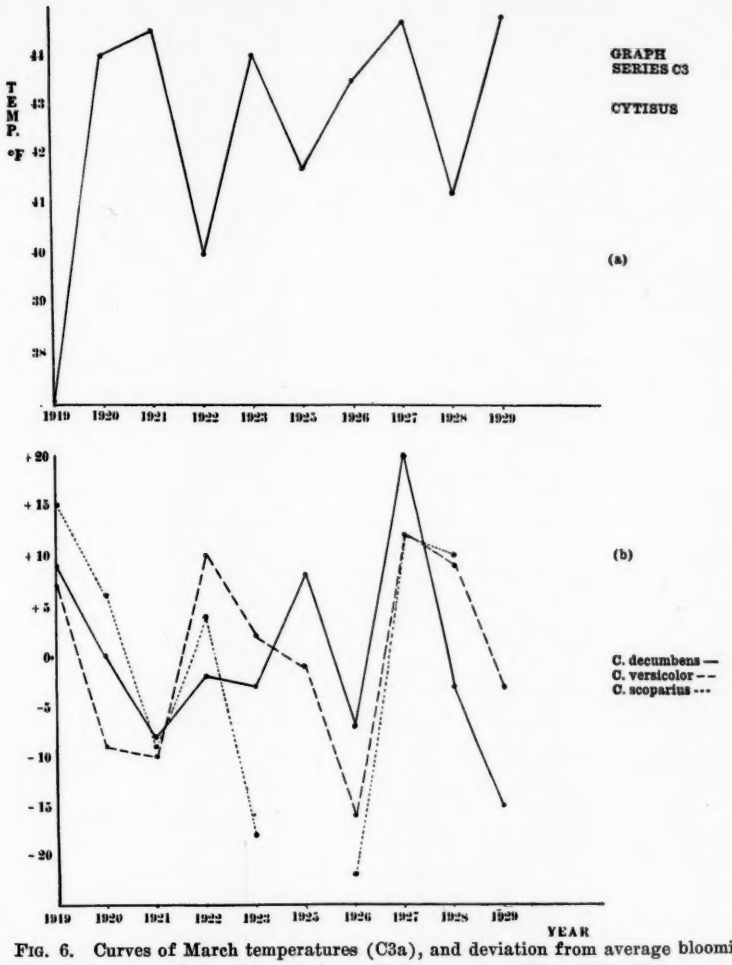

FIG. 6. Curves of March temperatures (C3a), and deviation from average blooming time for *Cytisus decumbens, C. versicolor,* and *C. scoparius* (C3b), 1919–1929.

	°F.
April 26	23
April 27	18
April 28	14.5
April 29	21
April 30	24
May 1	29

TABLE IV

Cytisus

A, DATE OF FLOWERING; B, DEVIATION FROM AVERAGE DATE OF FLOWERING

YEAR OF FLOWERING	AVERAGE TEMPERATURE (MARCH)	*C. kewensis*		*C. beanii*		*C. ardoinii*		*C. biflorus*		*C. purgans*		*C. decumbens*		*C. hirsutus*		*C. albus*	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1919	37.1	Apr. 26	+6	My. 10	+19	My. 3	+12	My. 10	+11	My. 17	+10	My. 17	+9	.....	.....	My. 10	−3
1920	44.0	Apr. 10	−10	Apr. 3	−18	Apr. 17	−4	Apr. 24	−5	Apr. 24	−13	My. 8	0	My. 22	+10	My. 1	−12
1921	44.5	Mar. 26	−25	Apr. 9	−12	Apr. 2	−19	Apr. 9	−20	Apr. 30	−7	Apr. 30	−8	Apr. 23	−19	Apr. 23	−20
1922	40.0	Apr. 29	+9	My. 13	+22	My. 6	+15	My. 13	+14	My. 20	+13	My. 6	−2	Ju. 3	+22	My. 20	+7
1923	44.0	Apr. 7	−13	Apr. 7	−14	Apr. 7	−14	Apr. 14	−15	Apr. 21	−16	My. 5	−3	My. 5	−7	Jn. 2	+20
1925	41.7	My. 2	+12	My. 2	+11	My. 2	+11	My. 9	+10	My. 2	−5	My. 16	+8	Jn. 6	+25	My. 25	+12
1926	43.5	My. 1	+11	Apr. 17	−4	Apr. 17	−4	My. 1	+2	My. 1	−6	My. 1	−7	Apr. 24	−18	My. 1	−12
1927	44.7	Apr. 23	+3	Apr. 9	−12	Apr. 23	+2	Apr. 30	+1	My. 14	+7	My. 28	+20	My. 14	+2	Apr. 23	−20
1928	41.2	Apr. 21	+1	Apr. 28	+7	Apr. 28	+7	My. 5	+6	.....	.....	My. 5	−3	My. 5	−7	Jn. 2	+20
1929	44.8	Apr. 27*	+3.5	Apr. 27*	+2.5	Apr. 27*	+2.5	Jn. 1*	−1.5	Jn. 1*	+21.5	Apr. 27*	−14.5	Jn. 1*	+16.5	Jn. 1*	+15.5

C. horniflorus		*C. versicolor*		*C. purpureus incarnatus*		*C. glabrescens*		*C. scoparius*		*C. scoparius prostratus*		*C. scoparius andreanus*		*C. purpureus albus*		*C. sessilifolius*		AVERAGE OF B COLUMNS
A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	A	
My. 31	+16	My. 24	+7	My. 31	+9	My. 17	−4	Jn. 7	+15	My. 24	+1	Jn. 14	+19	My. 31	+3	My. 31	+2	+8.25
Mar. 6	(−70)	My. 8	−9	My. 22	0	My. 8	−13	My. 29	+6	My. 8	−15	My. 22	−4	My. 22	−6	My. 29	0	−5.75
My. 7	−8	My. 7	−10	My. 14	−8	My. 7	−14	My. 14	−9	My. 28	+5	My. 7	−19	My. 14	−14	My. 14	−15	−13.00
Jn. 10	+26	My. 27	+10	Jn. 3	+12	My. 27	+6	My. 27	+4	Jn. 3	+11	My. 27	+1	Jn. 3	+6	Jn. 3	+5	+10.5
Jn. 23	+39	My. 19	+2	Jn. 2	−3	Jn. 16	+26	My. 5	−18	Jn. 2	+10	.....	.....	Jn. 2	+5	Jn. 2	+4	+0.25
My. 25	+10	My. 16	−1	Jn. 6	+3	Jn. 6	+16	.....	.....	My. 25	+2	My. 25	−1	Jn. 6	+9	Jn. 6	+8	+8.00
My. 1	−14	My. 1	−16	My. 22	−21	My. 1	−20	My. 1	−22	My. 1	−22	My. 29	+3	My. 22	−6	My. 15	−14	−10.00
.....	.....	My. 28	+11	My. 28	+6	My. 14	−7	Jn. 4	+12	My. 28	+5	My. 28	+2	My. 28	0	My. 28	−1	+2.00
.....	.....	My. 26	+9	Jn. 2	+4	My. 26	+5	Jn. 2	+10	My. 26	+1	Jn. 16*	+17.5	Jn. 2	+5	Jn. 16*	+14.5	+6.50
.....	.....	My. 18*	−2.5	Jn. 1*	−7.5	Jn. 1*	+7.5	.....	.....	.....	.....	Jn. 1*	+2.5	Jn. 1*	+0.5	Jn. 1*	−0.5	+4.00

* Record made at end of a fortnight.

Considering these data as a whole, it is clear that in *Cytisus* there are two belts of weather (proximal and immediate) during which temperature affects date of flowering. The mechanism through which the temperature operates is not precisely known, and must await a cytological investigation. It may be that the two periods synchronize with spore differentiation; the earlier period with microspore development, and the later with megaspore production. This implies the existence of a time interval between the two processes which remains to be proved. On the face of the data, it seems likely that there is no definite cessation of activity at an intermediate point, but rather that the two processes are continuous, the one following the other without a definite interval; but that this extended period offers an extended time for temperature to operate, similar in aggregate length to that in the species showing two definite periods of spore differentiation activity, and that within this aggregate period the temperature may influence the two significant processes. The fact that the sharp lines of spore differentiation activity are in this way somewhat blurred accounts for the apparently fickle nature of the genus as a whole.

TABLE V

Cytisus

A, average of deviations of all 17 species for year in question; B, average of deviations of all species except those late in 1923 (*C. albus*, *C. horniflorus*, *C. glabrescens*) for year in question; C, average of deviations of all species except those late in 1927 (*C. decumbens*, *C. versicolor*, *C. scoparius*) for year in question.

Year of flowering	Average temperature (March)	A	B	C
1919	37.1	+ 8.25	+ 9.50(1.25)*	+ 7.75(0.5)
1920	44.0	− 5.75	− 5.00(0.75)	− 7.00(1.25)
1921	44.5	−13.00	−12.75(0.25)	−14.00(1.0)
1922	40.0	+10.50	+10.00(0.5)	+12.00(1.5)
1923	44.0	0.00	− 6.25(6.25)	+ 1.50(1.5)
1925	41.7	+ 8.00	+ 7.00(1.0)	+ 8.75(0.75)
1926	43.5	−10.00	− 9.00(1.0)	− 9.00(1.0)
1927	44.7	+ 2.00	+ 4.00(2.0)	− 1.00(3.0)
1928	41.2	+ 6.50	+ 5.50(1.0)	+ 7.50(1.0)
1929	44.8	+ 4.00	+ 3.00(1.0)	+ 5.25(1.25)

* Figures in brackets indicate difference between this value and the value for A.

Syringa

When the table of deviations from average flowering date for this genus was drawn up, it showed a high degree of consistency for the genus as a whole. As in *Cytisus*, no distal temperature belt was discernible, neither

* Record made at end of a fortnight.

at first glance could a satisfactory proximal belt be found. The explanation was discovered in the marked overriding effect of the temperature at time of flowering (immediate temperature belt). When this belt was taken into consideration, *Syringa* was found to be governed, like *Cytisus*, by a proximal temperature belt in March, overridden by immediate temperature belts in April and May. Hence, although not at first evident, *Syringa* and *Cytisus* are very similar, although the immediate temperature belt has a more marked effect on *Syringa* than on *Cytisus*.

The twelve species of *Syringa* here considered fall into two classes according to their average time of flowering, hence in considering the genus two immediate temperature belts must be taken into account. The two species which have average flowering dates about the end of April/beginning of May (*S. villosa* var. *giraldii* and *S. pinnatifolia*) are governed by an immediate temperature belt in April. The other ten species, with average flowering dates at the end of May/beginning of June, are governed by an immediate temperature belt in May.

TABLE OF *SYRINGA* SPECIES

SPECIES	AVERAGE FLOWERING DATE
GROUP I:	
S. villosa giraldii	April 22
S. pinnatifolia	May 4
GROUP II:	
S. vulgaris	May 24
S. vulgaris alba	May 25
S. persica	May 25
S. villosa lutece	June 1
S. villosa	June 1
S. reflexa	June 6
S. yunnanensis	June 10
S. adamiana	June 13
S. emodii	June 17
S. emodii variegata	June 25

GROUP I: SPECIES FLOWERING IN LATE APRIL AND EARLY MAY

For these species March is the proximal temperature belt, and April the immediate temperature belt. Figure 7 and table VI refer to this group.

Examining figure 7, it will be seen that the curves of *S. villosa* var. *giraldii* and *S. pinnatifolia* (S1c), excluding *S. pinnatifolia* in 1923, follow the March temperature curve S1a rather consistently, the most significant deviations occurring in 1927 and 1929. Time of flowering in 1927, and

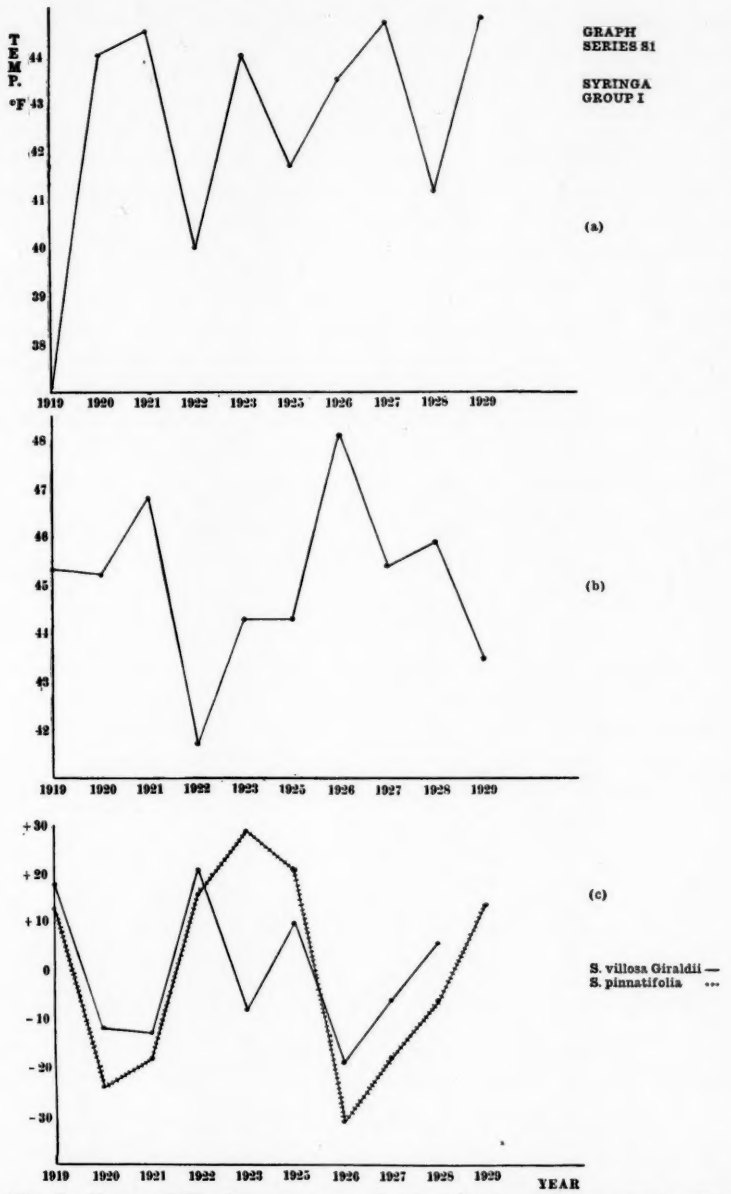

FIG. 7. Curves of March temperatures (S1a), April temperatures (S1b), and the deviation from average flowering time of *Syringa villosa giraldii* and *S. pinnatifolia* (S1c), 1919–1929.

more especially in 1929, was later than might be expected from the high temperature during March in those years. This is accounted for by the overriding effect of the April temperature (immediate belt), which in 1927 was average and in 1929 low. The only other years in which the April temperature curve (S1b) deviates from the March temperature curve (S1a) are 1919 and 1923. Comparison with the curve of flowering time (S1c) shows that in the former year April temperature has slightly modified the effect of March temperature. A point of more interest is the effect of the temperature in April, 1923, which was slightly below average. Apparently it had no effect on *S. villosa* var. *giraldii*, and a disproportionately powerful effect on *S. pinnatifolia*. A more detailed study of the weather conditions during April and May supplies the explanation. The temperature during the early part of April, although somewhat low, was not sufficiently low to retard the earlier flowering species *S. villosa* var. *giraldii*, which in consequence of a warm March flowered by April 14. Subsequent sharp frosts on April 15, 18, 27, and 28 checked *S. pinnatifolia* and held up its flowering so that it came under the influence of an extremely cold period which set in on May 9. Thus *S. pinnatifolia* in 1923 was four weeks late in flowering, and in this year should really rank as one of the later-flowering group with an immediate temperature belt in May.

TABLE VI

Syringa, Group I

Year of flowering	Average temperature		*S. villosa giraldii*		*S. pinnatifolia*	
	March	April	Date of flowering	Deviation from average	Date of flowering	Deviation from average
1919	37.1	45.3	May 10	+18	May 17	+13
1920	44.0	45.2	Apr. 10	−12	Apr. 10	−24
1921	44.5	46.8	Apr. 9	−13	Apr. 16	−18
1922	40.0	41.7	May 13	+21	May 20	+16
1923	44.0	44.3	Apr. 14	− 8	June 2	+29
1925	41.7	44.3	May 2	+10	May 25	+21
1926	43.5	48.1	Apr. 3	−19	Apr. 3	−31
1927	44.7	45.4	Apr. 16	− 6	Apr. 16	−18
1928	41.2	45.9	Apr. 28	+ 6	Apr. 28	− 6
1929	44.8	43.5	.....	.....	May 18	+14

Group II: Species flowering in late May and June

Here the proximal temperature belt is March and the immediate temperature belt May. Figure 8 and table VII refer to this group.

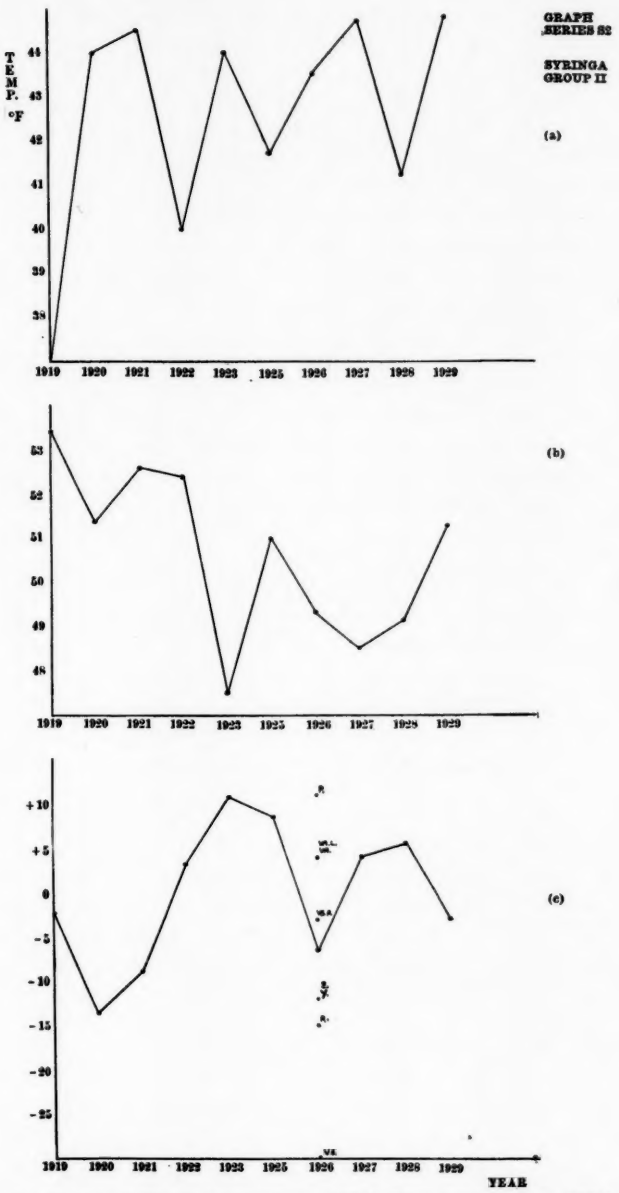

FIG. 8. Curves of March temperatures (S2a), May temperatures (S2b), and composite deviation from average blooming time for 10 species of *Syringa* (S2c), 1919–1929.

TABLE VII

Syringa, Group II

A, date of flowering; B, deviation from average date of flowering

Year of flowering	Average temperature		*S. vulgaris*		*S. vulgaris alba*		*S. persica*		*S. villosa lutece*		*S. villosa*	
	March	May	A	B	A	B	A	B	A	B	A	B
1919	37.1	53.4	May 24	0	May 24	−1	May 24	−1	.....	.....	May 31	−1
1920	44.0	51.4	May 22	−2	May 8	−17	May 15	−10	May 15	−17	May 15	−17
1921	44.5	52.6	May 14	−10	May 14	−11	May 14	−11	June 4	+3	May 14	−18
1922	40.0	52.4	June 3	+10	May 27	+2	May 27	+2	June 3	+2	June 3	+2
1923	44.0	47.5	June 9	+16	June 2	+8	June 2	+8	June 9	+8	June 9	+8
1925	41.7	51.0	June 6	+13	June 6	+12	May 25	0	June 6	+5	June 20*	+15.5
1926	43.5	49.3	April 24	−30	May 22	−3	June 5	+11	June 5	+4	June 5	+4
1927	44.7	48.5	June 4	+11	June 4	+10	May 21	−4	.....	.....	June 4	+3
1928	41.2	49.1	May 19	−5	May 26	+1	May 26	+1	.....	.....	.....	.....
1929	44.8	51.3	May 18	−6	June 1*	+3.5	June 1*	+3.5	June 1*	−3.5	.....	.....

	S. reflexa		*S. yunnanensis*		*S. adamiana*		*S. emodii*		*S. emodii variegata*		Average of B columns
	A	B	A	B	A	B	A	B	A	B	
1919	.....	.....	June 14	+4	June 14	+2	June 7	−10	June 14	−11	−2.25
1920	May 29	−8	May 22	−19	May 22	−21	May 29	−19	June 19	−6	−13.5
1921	.....	.....	June 4	−6	June 11	−1	June 4	−13	June 11	−14	−9.0
1922	June 10	+4	June 10	0	June 3	−9	June 24	+7	July 8	+13	+3.25
1923	June 9	+3	June 23	+13	June 30	+18	July 7	+20	June 30	+5	+10.75
1925	June 20*	+10.5	June 20*	+6.5	June 20*	+4.5	July 4*	+13.5	July 4*	+5.5	+8.5
1926	May 22	−15	May 29	−12	.....	.....	June 5	−12	.....	.....	−6.5
1927	June 11	+5	June 11	+1	June 11	−1	.....	.....	July 2	+7	+4.0
1928	June 16	+10	June 16	+6	June 16	+4	July 7*	+16.5	July 7*	+8.5	+5.25
1929	June 1*	−8.5	June 22†	.....	.....	.....	.....	.....	June 22†	.....	−2.5

* Record made at end of a fortnight.
† Record made at end of three weeks.

The composite graph of these ten later-flowering species (fig. 8, S2c) corresponds with the curve of March temperature (S2a); that is, of the proximal belt, except in 1923 and 1927. In 1923, March temperature was high but flowering was very late. In 1927, March temperature was also high, and flowering was again rather late. The graph of May temperature (S2b); that is, of the immediate belt, shows that the two lowest temperature records for that month occurred in the years 1923 and 1927, and may reasonably be taken to account for the deviations. In 1919, May temperature has modified but not overridden the effect of March temperature.

It must be noted that in 1926 there is indicated (fig. 8, S2c) a diversity in the behavior of the different species. With the available data it is not desirable to attempt to account for these, although the explanation offered in the case of *Cytisus* might be considered, namely, that a continuous advance of development through all the stages of spore formation exposes the plant for a longer time to action by external factors; and further, that at any "moment" in time the process will be more or less reactive to "momentary" fluctuations of the external factor. By momentary here is meant rather a short period, of one or two days at most.

Discussion

The foregoing data seem to indicate that the species of *Rhododendron, Cytisus,* and *Syringa* under consideration are affected as regards their date of flowering by the temperature at certain specified times during the year. While one species may in any particular year deviate from the general behavior of the natural group into which it falls here, nevertheless when all species are considered they show over a period of ten years a significantly consistent response to the temperature of distal and proximal, or proximal and immediate, weather belts.

It would seem that lower temperatures, obtaining during these significant though sometimes remote periods, delay the opening of flower buds; and conversely higher temperatures obtaining during the same periods in other years induce earlier flowering. The comparatively narrow belts of time during which deviations of temperature may be significant in inducing these effects are believed to synchronize with activity in spore formation in the plant. In other words, higher temperatures, occurring when there is activity in spore formation, lead to earlier flower burst; and lower temperatures, occurring at the same point, lead to later flower burst. The detailed mechanism through which such an effect may be brought about is deep seated and obscure. It would seem adequate simply to regard the higher temperatures as expediting the reactions involved in meiotic and premeiotic cell division; and there is no doubt that this must in large part be true.

† Record made at end of three weeks.

It is a matter of common observation and frequent record that different species of plants develop generally in definitely different temperature ranges, some plants requiring for normal growth an environment with a higher temperature range than others. Again some plants are more particular and demand a narrower range of temperature; and exposure to temperature below a minimum, higher than the minimum of other more "hardy" species, inhibits development and even causes death. These specific demands are, as has been said, well known although not well understood, and may be taken as the factor which conditions the "average" flowering date of different species.

Furthermore, the temperature demands of different organs of any one plant are not the same. In the great majority of cases in the annual history of the flowering plant vegetative activity occurs first in time, and usually at temperatures lower than will occur subsequently. The vegetative activity is followed by the change to reproductive phase, the change of phase being synchronized with onset of higher temperatures, so that in a scale of rising temperatures the reproductive phase comes as a culmination to the activity of the plant. Reverse cases are known, as for example *Tussilago farfara,* in which the reproductive phase is synchronized with the earlier period of lower temperatures, and flowering precedes vegetative activity. It may be that in the onset of the reproductive phase in such a genus as *Rhododendron,* where microspore and megaspore development take place in summer and spring respectively, this activity is dependent on temperature of narrow range, and may be inhibited, especially in the earlier flowering species, by higher as well as by lower temperatures.

While stress has been laid here on the connection between temperature belts and spore formation, it is not overlooked that the metabolic complex culminating in general development may be the fundamental cause of the fluctuations. It is significant that the localization in time has been related to a definite step in development.

Summary

Analyses of records on the date of flowering of various members of different genera at the Royal Botanic Garden, Edinburgh, show the following points:

1. The actual date of flowering of any species in any one year may vary from the average date.

2. Such aberration is referable to the temperatures obtaining during narrow belts of time.

3. These belts of time vary between genera as to the remoteness from the actual date of flowering, and here are referred to the periods of activity in gamete formation.

The writer is greatly indebted to Dr. ALEX. NELSON, Edinburgh University, for much help and encouragement during the course of the work, and to Professor W. WRIGHT SMITH and the staff of the Royal Botanic Garden, Edinburgh, for permission to use the data, and for other facilities.

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EFFECT OF FREQUENT CLIPPING ON THE DEVELOPMENT OF CERTAIN GRASS SEEDLINGS

JOSEPH H. ROBERTSON

(WITH TEN FIGURES)

Introduction

The study reported here deals with the effects of the removal of tops upon the development of various species of grass seedlings. Not only the establishment but also the yield and nutritive value of range and pasture grasses are dependent to a great extent upon the manner and frequency with which the tops are removed. Winter-killing and lowered vitality, with attendant decreased yield, result from improper utilization of the herbage. In native pastures and range lands, undesirable species increase when there is early grazing and overstocking (**8**). Although the relation of harvesting practices to the development of grassland is known in a general way from observation, and more definitely for a few species from experiment, there is still a definite need for further experimental work.

GRABER (**7**) has shown that frequent removal of tops limits root development. This in turn may lessen drought resistance and absorptive capacity, and result in increased injury from low temperature and insects. Since most of the foods used as reserves in roots are synthesized above ground, frequent harvesting necessarily limits these reserves. Internal food reserves as well as a supply of nutrients are factors in productivity. In addition, frequent removal of tops promotes a deficiency of nitrogen in the soil (**7**).

Dicotyledonous plants are similarly weakened by too early or too frequent harvesting. Pruning reduces the root spread of almond trees (**3**). Cutting of immature alfalfa increases invasion of weeds and may cause winter-killing. The specific gravity of alfalfa roots decreases slightly after each cutting. Cutting when the plants are mature gives more reserve food and higher yield with less winter-killing and a longer life of crop, whether grass or alfalfa (**1**). SAMPSON and MALMSTEN (**16**) found that harvesting range grasses twice each season increased the yield with but slight injury to the roots. Yield tends to vary inversely with frequency of clipping in bluestem pastures of Kansas. Disappearance of valuable species is proportional to frequency of cutting. The higher nutritive value gained from frequent harvesting does not compensate for loss in yield (**2**). The deleterious effect of frequent cutting may be offset partially by cutting the plants at a greater height above the ground (**2, 9, 18**). Reseeding of western range land is successful only when grazing is delayed until late in the season.

WOODMAN, NORMAN, and FRENCH (**20**) found that the nutritive value of pasture herbage, under fertilization and abundant rainfall, is independent of cutting periods. Increased carrying capacity, under rotational grazing as compared with continuous grazing, was in the ratio of 3:2.

Many of the trials made recently show that the behavior of grasses following clipping is largely influenced by diminished food reserves and retarded development of roots. Normal root growth is rhythmic and, on the basis of dry weight, may be plotted as a series of SACHS grand-period-of-growth curves (**15**). This rhythm is disturbed on removal of tops.

Any dwarfing in the root system of a seedling may be regarded as serious. When the water relation is controlling, the fate of a seedling often depends upon its ability to develop an adequate root system. Under competition an added inch of growth underground may enable the plant to overtop its neighbors, the result often being the difference between survival and death. Extent of roots, rate of growth, and degree of tillering all are closely correlated (**19**). Seedlings of grasses are susceptible to injury from drought, erosion, and trampling, and their resistance to these is due in a great measure to the development of an adequate root system. Plants with larger root systems, either in soil or water cultures, produce more tillers upon addition of nitrogen than do those with smaller roots (**6**). JACKSON (**10**) found that nodal roots are always present at the beginning of tillering in barley. In general, higher yielding species of grasses have heavier root systems (**17**).

There is almost unanimous agreement that the best development of roots and tops goes hand in hand. An exceptional finding is that of LAIRD (**12**), who states that "the largest and deepest root systems of sod-forming grasses are not necessarily associated with the best and most vigorous top growth. Mowing of Centipede and Bermuda grasses increased the root growth" as measured by dry weight. STAPLEDON and BEDDOWS (**17**) compared the yield of normal plants with the bulk of their individual root systems and found no definite correlation.

According to PARKER and SAMPSON (**13**), frequent cutting produces measurable effects upon anatomical structure. Diameters of both root and stele as well as the number of ducts were decreased as a result of clipping. Similar results have recently been obtained by BISWELL (**4**).

Experimentation

The seeds of four native prairie grasses were used. They were bluegrass (*Poa pratensis*), blue grama grass (*Bouteloua gracilis*), June grass (*Koeleria cristata*), and needle grass (*Stipa spartea*). The seeds were collected at maturity near Lincoln, Nebraska, in 1931. Seeds of Hungarian brome grass (*Bromus inermis*) and Sudan grass (*Holcus sorghum sudanensis*), both exotic pasture grasses, were purchased at a local seed store.

The soil used was fertile loess having a hygroscopic coefficient of 8.9 per cent., a humus content of 7.6 per cent., and a circumneutral reaction. After screening the soil, sufficient water was added to increase its moisture content to about 30 per cent., which was approximately optimum for growth.

Forty-eight galvanized iron containers were used, ranging from 8 to 24 inches in depth and from 16 to 72 square inches in cross-sectional area. They were filled on October 16, the soil being uniformly compacted in all. Seeds of each of the six species of grasses were then planted, eight containers being used for each species. Four containers for each species were labeled 1, 2, 3, and 4, and the four remaining ones 1A, 2A, etc. The shallower containers were used for the plants that were to be grown only a few weeks; the deeper ones for the longer periods of growth. Thus the plants had, with few exceptions, ample room for normal depth of root development. The weight of each filled container was recorded.

With the exception of those with bluegrass, the containers were kept covered for six days following planting. Light sprinkling for a period of ten days insured rapid germination. Seven days after planting, the containers were restored to the original weight by adding water, and the heights of the seedlings were measured. This was repeated every seventh day during the experiment. The containers were also turned and shifted at these times to insure equal reception of light.

When a good stand was assured, brome grass and grama grass were thinned to 25 plants per container; bluegrass and June grass to 50 plants; and the coarser Sudan grass and needle grass to 12 plants.

Since the work was done during winter, sunlight was supplemented by two 150-watt electric lights. They were mounted in 15-inch white enameled reflectors suspended 3 feet above the tops of the containers. These lights were employed from 5:30 P. M. to 8:00 P. M., October 23 to February 18. In addition, they were used during cloudy weather. Measurements of light intensity, taken on January 17 at 12:00 M. with a Weston photronic cell and milliammeter, gave a ratio for outdoor direct sunlight to sunlight in the greenhouse of 7:6. The ratio for artificial light at 8:00 P. M. to sunlight at noon in the greenhouse was 1:15 directly under the reflector and 1:90 at the tops of the containers farthest from the source of light.

It was decided that under the conditions of this experiment, grazing could be represented best by removing the tops of the grasses by clipping. Accordingly, when each species reached a height suitable for grazing, the plants in containers labeled 1A, 2A, etc., were clipped. Sudan grass was clipped 3 cm. above the soil surface and the other grasses 1.5 cm. The tops were placed in weighing bottles and dried in an electric oven for 24

hours at 75° C. After being cooled in a desiccator they were weighed to the nearest 0.01 gm.

When the clipped plants had again reached sufficient height for grazing, tillers were counted and the tops again removed and weighed. This was also done with the unclipped plants of each species in containers no. 1. The soil was then carefully washed away from the roots in containers 1 and 1A. This was accomplished by placing the containers in a slanting trough and gently playing a small stream of water on the soil. The escaping muddy water was run through a small-meshed sieve and the few root ends that were broken off were recovered. These were included in the measurements of weight and volume.

Comparisons of roots included average length, volume, dry weight, and anatomical structure. Volume was found by displacement of water in a cylindrical graduate after the water on the surface of the roots had been removed by pressing them between paper towels. Portions of adventitious roots, 2.5 to 4 cm. from their tips, were fixed in chromo-acetic acid solution. Transverse sections of these were cut 15 μ thick and compared as to internal structure.

Individual root systems of Sudan grass and needle grass were separated from the mass of roots after they were placed in the shallow pans and floated in water. Root systems of average size were selected. Black paper, against which the light-colored roots showed plainly, was cross-ruled with white lines and fastened in the bottom of the pan, which was then covered with 1.5 inches of water. The root systems were floated out to their natural positions and drawn life-size on cross-ruled paper.

When the clipped plants had again reached their former height, the tops were clipped a third time and comparisons of roots and shoots of plants in containers 2 and 2A were made. This process was continued until the last lot of plants was examined on February 26.

Because of differences in rate of growth and in ability to recover after clipping, as well as variable periods of cloudiness, it was necessary to vary the length of intervals between clippings.

The temperature of the greenhouse averaged 23° C. but varied from 15° to 30° during the eighteen weeks of the experiment. The humidity at all times was rather high. Soil samples taken from containers showed that the variation in moisture content was between 21.6 and 30.4 per cent.

An attack of aphids made it necessary to spray the plants on January 15. The Sudan grass was injured to some extent by the sulphur-tobacco mixture.

Results

Bouteloua gracilis

The grama grass was clipped when five weeks old and at 21-day intervals thereafter. At the time of the first clipping it was 8 cm. tall and had

three leaves per plant. Thus it had made an average daily growth, after appearing above ground, of 2.6 mm.

A comparison of growth rates after each clipping shows that the first and third treatments inhibited elongation slightly while the second and fourth stimulated it somewhat. The total result of the four clippings was a slight inhibition. The total increase in height during the seventeen weeks was 23 cm. for the clipped and 24 cm. for the unclipped plants.

Six leaves were produced by the control and four by the clipped plants during the period of the experiment. Tillers were ten times as numerous and sixteen times as tall on the unclipped as on the clipped plants (table I).

Dry weight of tops decreased after the first cutting and increased slightly after each of the last three cuttings (fig. 1). The initial decrease may have been due to shortening days, November 20 to December 11, coincident with 73 per cent. cloudiness. The total dry weight of the clipped tops was 0.15 gm. The tops of the control plants weighed 2.46 gm., or over sixteen times as much.

A marked difference in growth of roots was found. While the roots of the control plants penetrated continuously to a depth of 33 cm., those of the clipped plants decreased with each removal of tops (fig. 1). Neither the dry weight nor the volume of the roots increased markedly after the first clipping. The final dry weight ratio was 1:35 for roots of clipped and unclipped plants respectively. That of volume was 1:52 (table I).

The diameters of the roots of clipped and unclipped plants were in the ratio of 3:4. Fifty per cent. of the diameter of the clipped plants consisted of stele while in the unclipped ones the stele composed only 46 per cent. Xylem strands were more abundant in the latter (table II).

Table I gives the percentage of survival after each of the cutting treatments.

Bromus inermis

Clipping was begun 28 days after planting, when the seedlings were 13 cm. tall and in the third-leaf stage. They had grown at the rate of 5 mm. per day after germination. During the 13 weeks the clipped plants elongated 10 cm. more than the unclipped ones. Thus clipping resulted in a 23 per cent. increase in height.

At the end of the experiment the undisturbed plants had eight leaves while those clipped four times had five. The clipped plants did not tiller. An average of six tillers 25 cm. tall was produced by the control plants (table I). The final leaf widths were 2.4 mm. and 5.8 mm. for the clipped and unclipped plants respectively.

Weight of tops decreased after each clipping (fig. 2). The five separate cuttings yielded 4 per cent. as much dry weight as the one final cutting.

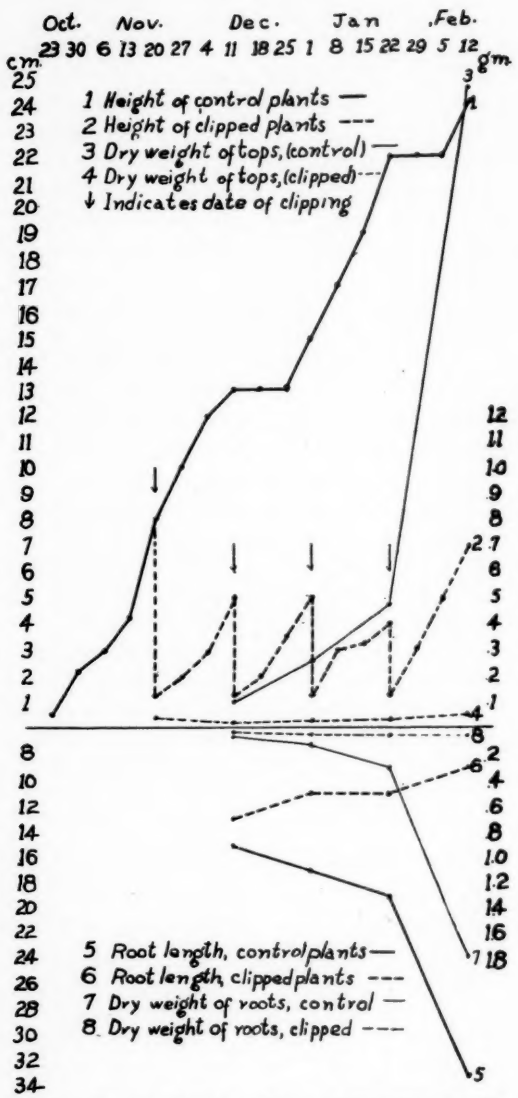

Oct. Nov. Dec. Jan Feb.
23 30 6 13 20 27 4 11 18 25 1 8 15 22 29 5 12
cm.
gm.
1 Height of control plants
2 Height of clipped plants
3 Dry weight of tops, (control)
4 Dry weight of tops, (clipped)
↓ Indicates date of clipping
5 Root length, control plants
6 Root length, clipped plants
7 Dry weight of roots, control
8 Dry weight of roots, clipped

FIG. 1. Growth of roots and tops of *Bouteloua gracilis*.

TABLE I

DATA ON TOP AND ROOT DEVELOPMENT NOT INCLUDED IN FIGURES 1–10. FIGURES BASED ON CONTAINERS REMOVED ON DATES INDICATED

SPECIES	DATE OF CLIPPING	NUMBER OF LEAVES		TILLERS				ROOT VOLUMES		SURVIVAL PERCENTAGE	
				NUMBER		HEIGHT					
		UNCUT	CUT	UNCUT	CUT	UNCUT	CUT	UNCUT	CUT	UNCUT	CUT
						cm.	*cm.*	*cc.*	*cc.*	%	%
Bouteloua gracilis	Dec. 11	5.0	2.5	0.0	0.0	0.0	0.0	0.3	0.2	100	100
	Jan. 1	5.0	3.6	0.7	0.0	4.0	0.0	0.5	0.1	100	80
	Jan. 22	5.0	3.0	2.1	0.2	8.0	2.0	1.5	0.3	100	72
	Feb. 12	6.0	4.0	5.0	0.5	16.0	1.0	10.5	0.2	100	92
Bromus inermis	Dec. 2	6.0	4.0	0.5	0.1	1.3	0.2	5.5	2.3	100	100
	Dec. 18	6.0	5.0	0.5	0.0	8.0	0.0	13.0	1.8	100	100
	Jan. 3	7.0	5.0	2.5	0.0	4.0	0.0	23.0	1.7	100	100
	Jan. 18	8.0(2)*	5.0	6.3	0.0	25.0	0.0	56.0	0.8	100	68
Holcus sorghum sudanensis	Dec. 2	5.0	3.0	2.0	0.2	5.0	3.0	5.0	1.1	100	100
	Dec. 18	5.5	3.0	2.8	2.0	11.0	5.5	11.6	1.4	100	83
	Jan. 3	8.0	3.0	6.0	1.0	31.0	7.0	43.5	0.8	100	83
	Jan. 18	8.0(3)	3.0	11.0	1.5	41.0	6.0	62.0	1.5	100	75
Koeleria cristata	Dec. 23	5.5	4.0	0.8	0.2	2.0	0.5	1.2	0.3	100	100
	Jan. 11	6.0	4.0	1.0	0.5	1.5	0.4	4.8	0.7	100	100
	Jan. 30	6.0(2)	4.0	3.0	0.1	7.0	1.0	3.7	0.9	100	100
	Feb. 20	7.0(2)	5.0	2.5	1.4	8.0	3.0	8.0	2.9	100	100
Poa pratensis	Dec. 9	6.0	3.0	1.0	0.0	5.0	0.0	13.0	8.5	100	100
	Dec. 23	6.0	3.0	1.5	0.0	2.0	0.0	29.0	11.0	100	100
	Jan. 11	7.0	3.0	1.1	0.0	11.5	0.0	25.0	15.0	100	100
Stipa spartea	Dec. 9	3.0	2.0	0.0	0.0	0.0	0.0	2.1	1.6	100	100
	Jan. 6	5.0(1)	2.0	0.2	0.0	15.0	0.0	2.5	0.4	100	65
	Feb. 3	5.0(1)	2.0	2.0	0.0	23.0	0.0	15.3	0.7	100	75
	Feb. 26	5.0(1)	2.0	3.0	0.0	30.0	0.0	20.5	1.1	100	75

* Figures in parentheses indicate number of dead leaves.

TABLE II

EFFECT OF FOUR CLIPPINGS ON ANATOMICAL STRUCTURE OF NODAL ROOTS. DATA BASED ON TRANSVERSE SECTIONS 15 μ THICK, FROM PORTIONS OF ROOTS 2.5 TO 4 CM. FROM TIPS

SPECIES	TREATMENT	DIAMETER OF ROOT	DIAMETER OF STELE
		μ	μ
Bouteloua gracilis	Clipped	252	126
	Unclipped	332	151
Bromus inermis	Clipped	189	113
	Unclipped	490	215
Holcus sorghum	Clipped	248	126
	Unclipped	549	312
Koeleria cristata	Clipped	262	119
	Unclipped	216	91
*Poa pratensis**	Clipped	119	66
	Unclipped	329	112
Stipa spartea	Clipped	307	134
	Unclipped	472	168

* After two clippings.

Root penetration was practically stopped by clipping. Increase in length was slow until the fourth cutting, which was followed by a slight decrease. The unclipped plants had roots which were nearly four times as long as those of the clipped ones (figs. 2, 3). The ratio for the dry weight of roots was 1:51 for the clipped and unclipped plants respectively. The corresponding volume ratio was 1:70.

A response of brome grass to removal of tops was observed by growing seedlings in soil in glass jars 2 feet deep. These were tilted at an angle of 20° from the vertical. Rate of growth down the side of the jar was observed to diminish gradually as a result of clipping. Growth then ceased entirely for 12 days before the roots started to die back from the tips. The rate at which they died back was approximately the same as that at which they had penetrated before clipping was begun.

The diameters of the roots of cut and uncut plants were in the ratio of 3:8. The stele of the treated plants made up 59 per cent. of the diameter as compared with 43 per cent. for the untreated ones. Metaxylem elements were larger and more than twice as numerous in the uncut plants.

Survival fell to 68 per cent. after the fourth cutting (table I).

HOLCUS SORGHUM SUDANENSIS

The seedlings of the Sudan grass were first clipped 4 weeks after planting. At that time they were 13 cm. tall and in the late fourth-leaf stage.

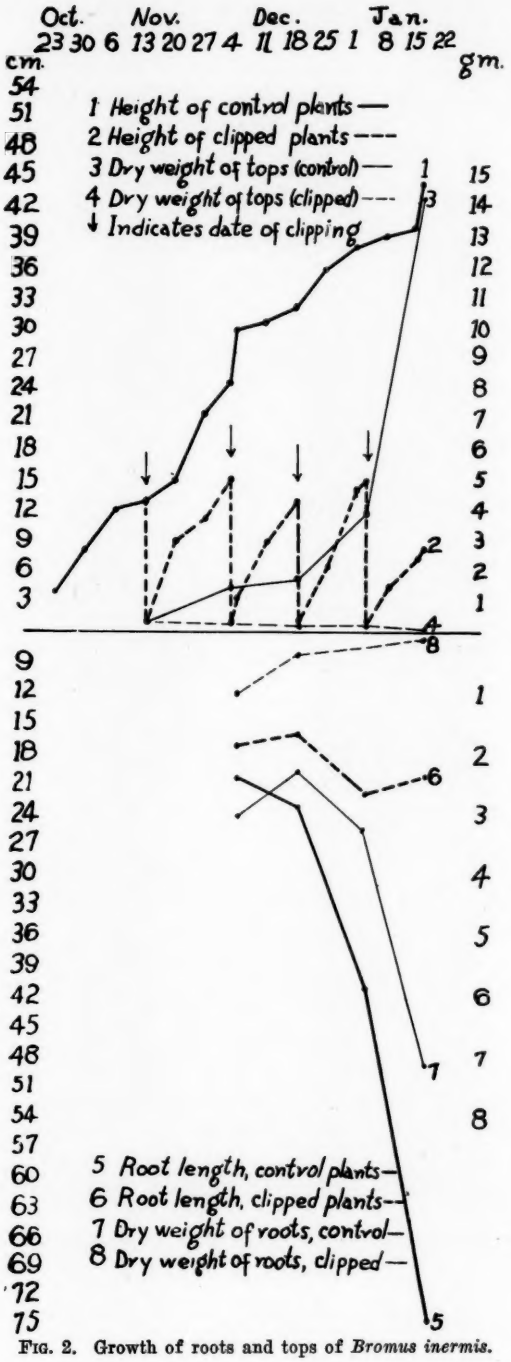

Oct. Nov. Dec. Jan.
23 30 6 13 20 27 4 11 18 25 1 8 15 22
cm.
gm.
1 Height of control plants —
2 Height of clipped plants ---
3 Dry weight of tops (control) —
4 Dry weight of tops (clipped) ---
↓ Indicates date of clipping
5 Root length, control plants —
6 Root length, clipped plants --
7 Dry weight of roots, control —
8 Dry weight of roots, clipped —

FIG. 2. Growth of roots and tops of *Bromus inermis*.

FIG. 3. Unclipped and clipped *Holcus* (left) and *Bromus* (right). 1/11 natural size.

After every clipping the unclipped plants elongated more rapidly than the clipped ones. During nearly half of the experiment the rate of elongation of the cut plants was less than one-half as rapid as that of the controls. The total increase in height of the clipped plants was 39.5 cm.; the final height of the unclipped ones was 60 cm.

By the end of the 13 weeks, the unclipped plants had eight leaves but the clipped ones only three. Tillers were over seven times as numerous and nearly seven times as tall on the unclipped as on the clipped ones (table I).

Dry weight of tops decreased after the first and second clippings but increased slightly after the third and fourth, probably as a result of difference in length of day (fig. 4). The ratio of total dry weight was 1:14 for clipped and unclipped plants respectively. The final leaf widths were 6.1 mm. on the clipped and 13.6 mm. on the unclipped plants.

Differences in length of roots increased after each treatment. The roots of the clipped plants increased in length until the fourth clipping and then decreased (fig. 4). Sixteen days after the first clipping, the roots of the clipped plants were three-fifths as long as those of the unclipped. At the same length of time after the fourth cutting, the ratio was 3:7. The dry weight ratio at the end of the experiment was 1:40 and the volume ratio 1:41 for the clipped and unclipped plants respectively.

The clipped plants were characterized by short adventitious roots and nearly normal primary roots (figs. 5, 6). The adventitious roots began to die back from the tips after the second clipping. The plants apparently maintained a certain balance between development of roots and tops. Production of tillers and growth of adventitious roots correlated closely (table I; figs. 5, 6).

Roots of the control plants were more than twice as great in diameter as those of the clipped ones. Fifty-one per cent. of the diameter of the latter was stele, as was 57 per cent. of the diameter of the roots of the control plants (table II).

Survival fell to 83 per cent. after the second clipping and to 75 per cent. after the fourth (table I).

Koeleria cristata

On December 4 the seedlings of the June grass had four leaves each and were 6.5 cm. tall. This amounted to 1.4 mm. growth daily since germination. The plants were clipped at this date and four times thereafter at intervals of 21 days. The tops of the clipped plants elongated faster than those of the unclipped throughout the period of the experiment. That the growth of these plants was stimulated by cutting is shown further by the fact that the control plants attained a height of only 15.5 cm. while the total increment of the clipped ones was 19.5 cm.

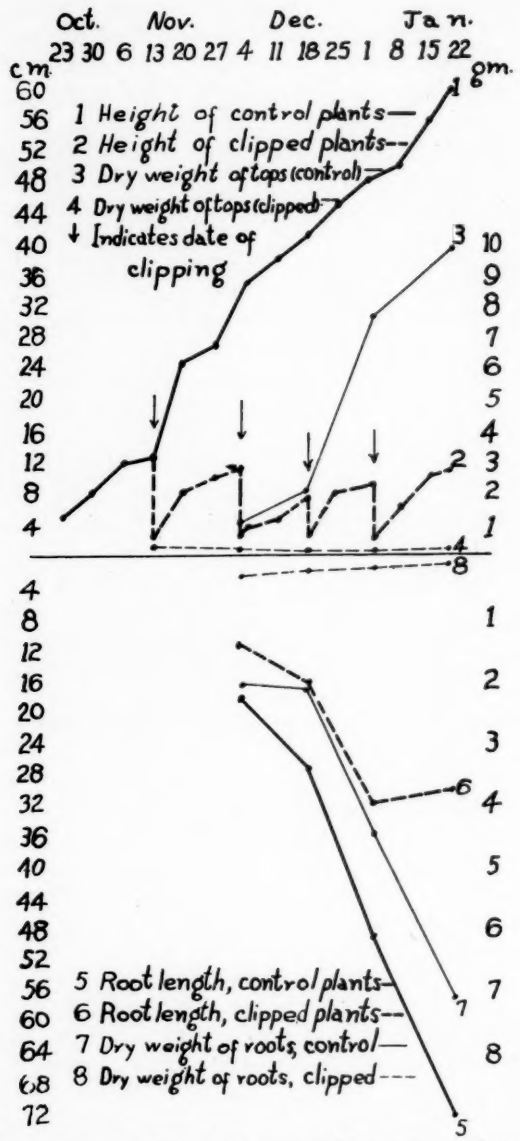

Oct. Nov. Dec. Jan.
23 30 6 13 20 27 4 11 18 25 1 8 15 22
cm.
gm.
1 Height of control plants
2 Height of clipped plants
3 Dry weight of tops (control)
4 Dry weight of tops (clipped)
↓ Indicates date of clipping
5 Root length, control plants
6 Root length, clipped plants
7 Dry weight of roots, control
8 Dry weight of roots, clipped

FIG. 4. Growth of roots and tops of *Holcus sorghum sudanensis*.

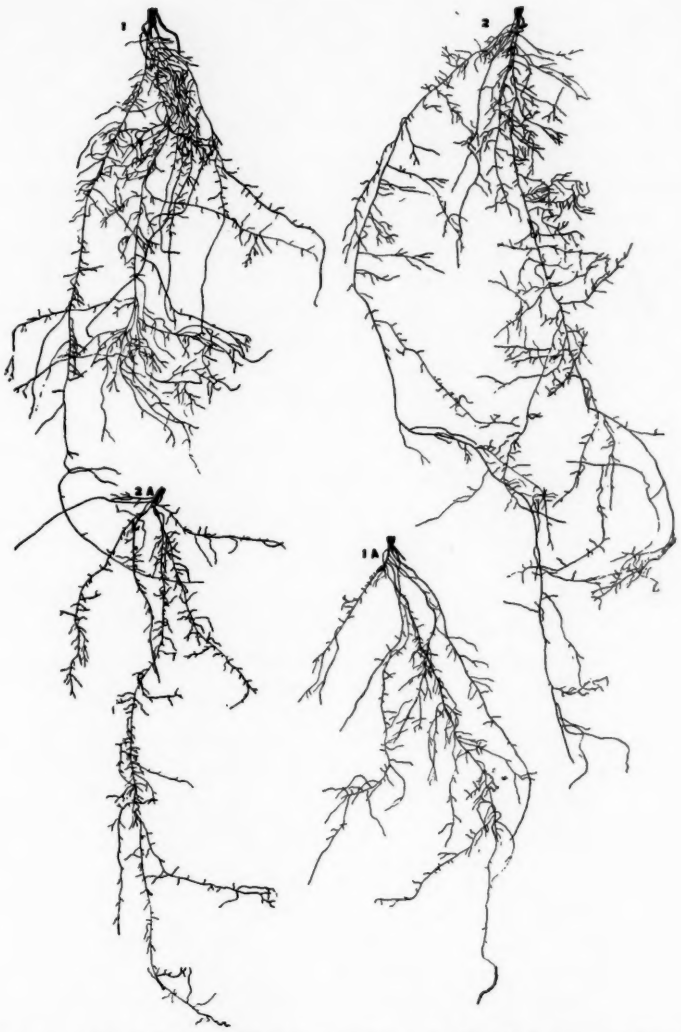


FIG. 5. Roots of *Holcus sorghum sudanensis:* 1, unclipped; 1A, clipped once (both 7 weeks old); 2, unclipped; 2A, clipped twice (both 9 weeks old). About ¼ natural size.

The plants clipped four times had an average of five leaves; those not cut had seven. Tillers were nearly twice as numerous on the control plants and nearly three times as tall as on the clipped ones (table I).

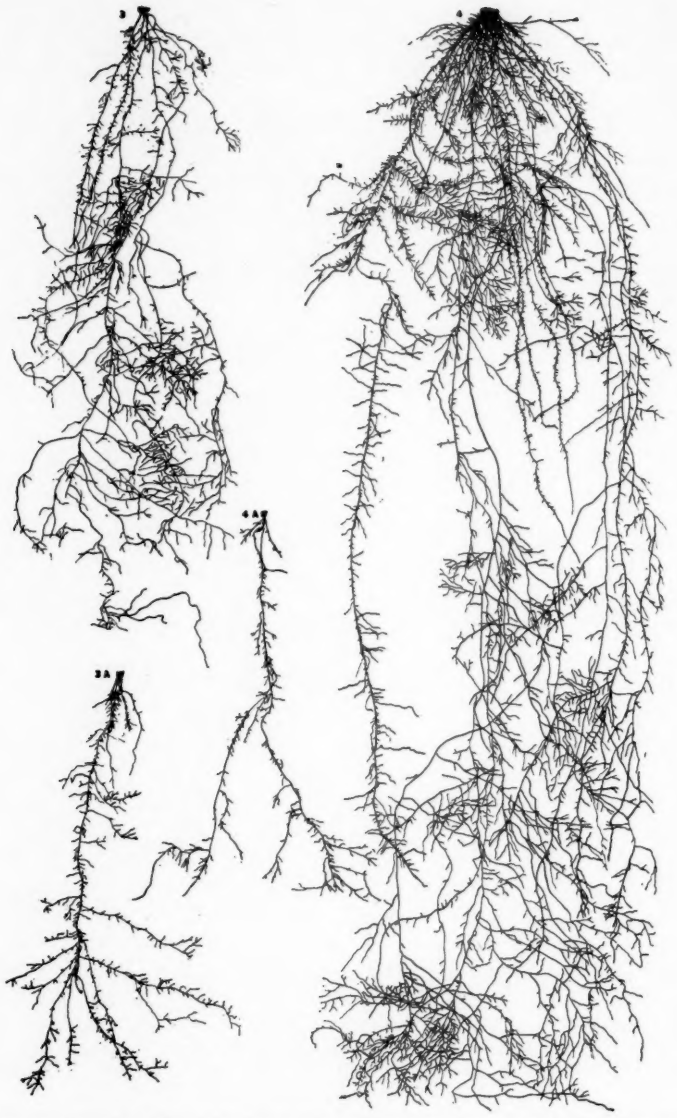


FIG. 6. Roots of *Holcus sorghum sudanensis:* 3, unclipped; 3A, clipped three times (both 11 weeks old); 4, unclipped; 4A, clipped four times (both 13 weeks old). About ⅙ natural size.

Each of the first four cuttings yielded approximately the same weight of dry matter. The fifth cutting showed considerable increase (fig. 7). Evidently the interval was sufficient to permit recovery as the days lengthened from January 30 to February 20. The five cuttings yielded nearly one-fifth as much as the one final cutting, which was much greater than for any of the other clipped grasses. This correlated with the relatively small differences in number and width of leaves. The number of living leaves (five) was the same in each at the end of the experiment. Those of the control plants were 1.6 mm. wide; the clipped ones were 1.5 mm. in width.

Root penetration continued in spite of clipping (fig. 7). The cutting treatment retarded growth an average of only 1.0 mm. per day throughout the experiment. The effect of clipping becomes more apparent when the weights of the roots are compared (fig. 7). Clipping reduced the weight of roots to one-third that of the control. The corresponding volume ratio after 18 weeks was 10:36.

Unlike the other species, the June grass responded to clipping by producing roots of greater diameter, the ratio being 6:5 for cut and uncut plants (table II). All of the plants survived the treatment (table I).

Poa pratensis

At the time of the first clipping, November 20, the bluegrass seedlings were 9 cm. high and in the fourth-leaf stage. The rate of growth, as measured in mm. per day, was reduced to a slightly different extent by each clipping treatment. On an average, clipping inhibited growth 0.5 mm. per day.

The clipping treatment permitted the formation of only three leaves while the control plants developed seven. Those of the clipped plants were 1 mm. wide as compared with 1.8 mm. for the unclipped ones. Clipping prevented tiller production (table I).

Weight of tops decreased until the third clipping, after which it increased (fig. 8). The tops produced by the control plants during the 12 weeks weighed 7.6 times as much as those of the clipped ones.

The roots made a definite response to each removal of photosynthetic area. Nineteen days after the first clipping, the weights of the roots of clipped and control plants were in the ratio of 1:4. This ratio was reduced to 1:11 by the second and 1:14 by the third treatment.

Root penetration continued in spite of clipping but was less rapid than in the control plants (fig. 8). The roots of the seedlings which were clipped three times were only three-fifths as long as those of the unclipped ones. Stated differently, clipping inhibited root penetration an average of 2 mm. per day.

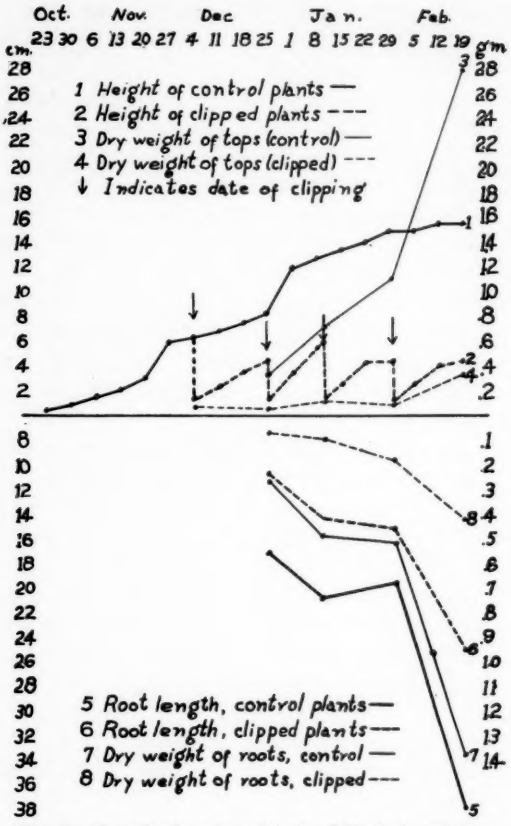

Oct. Nov. Dec Jan. Feb.
23 30 6 13 20 27 4 11 18 25 1 8 15 22 29 5 12 19
cm.
gm.
1 Height of control plants
2 Height of clipped plants
3 Dry weight of tops (control)
4 Dry weight of tops (clipped)
↓ Indicates date of clipping
5 Root length, control plants
6 Root length, clipped plants
7 Dry weight of roots, control
8 Dry weight of roots, clipped

FIG. 7. Growth of roots and tops of *Koeleria cristata*.

The ratios of root volumes of clipped and unclipped plants became smaller with each treatment. They were 1:7, 1:15, and 1:20 after the first, second, and third cuttings.

As a result of two clippings, the diameter of the adventitious roots was reduced to about one-third that of the uncut plants. In the latter the stele formed 34 per cent. of the root diameter, while in the cut plants it constituted 55 per cent. (table II).

STIPA SPARTEA

The seedlings of needle grass were first clipped on November 20. On this date they had three leaves and were 12 cm. in height. The first and

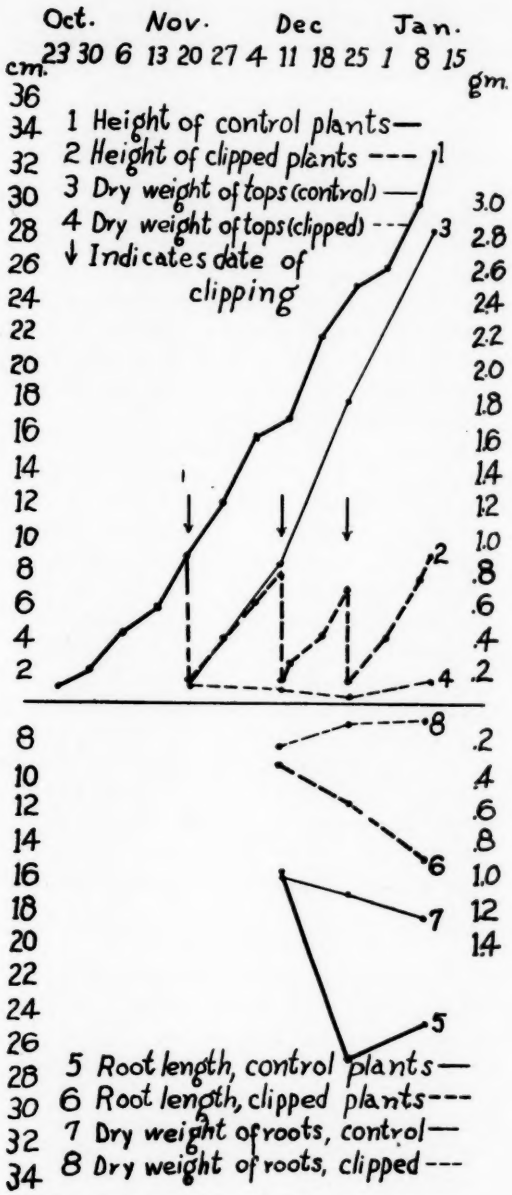

Oct. Nov. Dec Jan.
23 30 6 13 20 27 4 11 18 25 1 8 15
cm.
gm.
1 Height of control plants—
2 Height of clipped plants ---
3 Dry weight of tops (control) —
4 Dry weight of tops (clipped) ---
↓ Indicates date of clipping
5 Root length, control plants—
6 Root length, clipped plants---
7 Dry weight of roots, control—
8 Dry weight of roots, clipped ---

FIG. 8. Growth of roots and tops of *Poa pratensis*.

second clippings apparently inhibited elongation of tops. After the third treatment no difference in rates of growth due to clipping was detected. The controls elongated only 28 per cent. as much as the cut plants after the fourth treatment. Hence clipping apparently caused a 30 per cent. increase in height.

Cutting limited the number of leaves to two while the control plants developed five. The control plants produced three tillers 30 cm. tall. Clipping entirely prevented the production of tillers (table I).

Weight of tops decreased slightly after the first and second cuttings and a small increase was evident after the third and fourth (fig. 9). These increases were probably due to the greater length of day, January 6 to February 26. The five cuttings yielded less than 7 per cent. as much dry weight of tops as one final cutting. Leaves of the clipped plants were only 0.92 mm. wide as compared with 2.53 mm. for those of the control plants.

The effect of each clipping was strongly reflected by the roots. As in the case of Sudan grass, the chief difference was in the checked development of adventitious roots (fig. 10). Development of these roots was prevented by the first three cuttings. Their growth after the fourth cutting was evidence of recovery. With but one exception, root penetration continued after each clipping; but the weight decreased until the fourth clipping, after which a slight increase was made (fig. 9). At the conclusion of the experiment the control plants had roots which were 2.6 times as long as those of the clipped ones. Clipping was thus responsible for retarding root penetration to an average extent of 2.7 mm. daily during the 130 days.

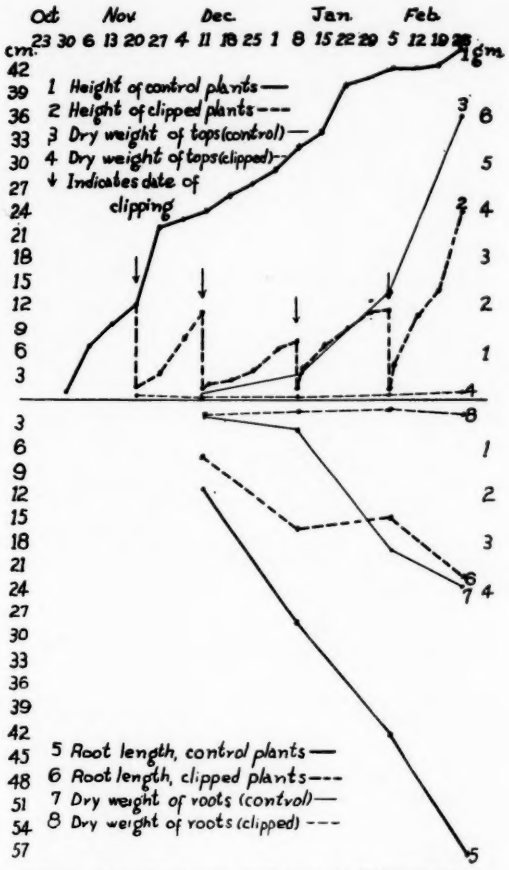
The volume ratio of the roots of unclipped plants to those of plants clipped four times was 18 : 1. The corresponding weight ratio was 13 : 1.

The roots of the clipped plants were only two-thirds the diameter of those of the unclipped ones. In the clipped plants the stele made up 44 per cent. of the root diameter, while in the control plants it composed only 35 per cent.

Seventy-five per cent. of the plants survived the four treatments.

Discussion

This experiment shows clearly that the development of grass seedlings is seriously affected by frequent removal of tops. Certain species, such as *Koeleria,* are injured much less than others, such as *Stipa* and *Holcus.* GRABER (7) reports that nine cuttings injured alfalfa more than twenty-two cuttings did bluegrass. Differences in response are shown also by the fact that elongation of tops in three of the grasses (*Poa, Bouteloua,* and *Holcus*) was inhibited, while in the other three (*Bromus, Koeleria,* and *Stipa*) it was slightly stimulated by clipping.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cm.
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1 Height of control plants
2 Height of clipped plants
3 Dry weight of tops (control)
4 Dry weight of tops (clipped)
↓ Indicates date of clipping
5 Root length, control plants
6 Root length, clipped plants
7 Dry weight of roots (control)
8 Dry weight of roots (clipped)

FIG. 9. Growth of roots and tops of *Stipa spartea*.

All of the species responded to clipping by reduction in yield and decreased development of roots. This was effected through reduction in number and height of tillers, number and width of leaves, and number and length of roots. The close relation in *Stipa* and *Holcus* between the production of tillers and nodal roots is in accord with the finding of KRASSOVSKY (**11**) for wheat and barley.

The nodal roots were the underground parts chiefly affected. In *Stipa* these roots were not produced until after the fourth cutting of tops. The tops also showed marked recovery at this time. The nodal roots of *Holcus* were produced early in the experiment and were reduced gradually by the

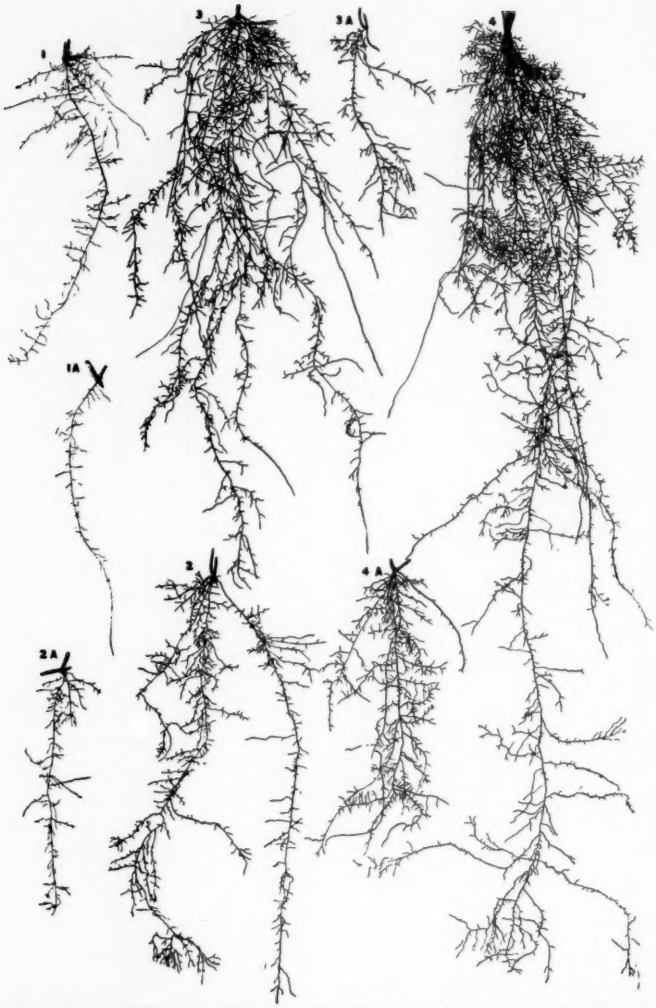

FIG. 10. Roots of *Stipa spartea:* 1, unclipped; 1A, clipped once (both 8 weeks old); 2, unclipped; 2A, clipped twice (both 12 weeks old); 3, unclipped; 3A, clipped three times (both 16 weeks old); 4, unclipped; 4A, clipped four times (both 19 weeks old). Slightly less than ⅓ natural size.

clipping treatment. Clipping retarded the roots of *Bouteloua* to one-fifth the length of the controls. Roots of *Koeleria* reached about five-eighths their normal length.

The mass of the roots, based on dry weight, decreased or remained constant except for *Koeleria,* which increased in spite of clipping. *Bromus* suffered the greatest reduction in both tops and roots on the basis of dry weight. The tops of the clipped plants weighed one-twenty-fifth and their roots only one-fiftieth as much as those of the controls. The corresponding ratios for *Holcus* were 1:14 and 1:40. In *Stipa* the reduction of tops was approximately the same as that of roots, being 1:14 and 1:13. The roots of the clipped *Poa* were one-fourteenth and the tops one-eighth as heavy as those of the unclipped plants. *Koeleria* was injured the least as measured by reduction in weight of tops and roots.

In spite of clipping, penetration continued slowly in all species except *Bouteloua,* which responded by a gradual decrease in root length. It is significant that the roots of *Bromus* ceased growth for 12 days before starting to die back from the tips following repeated removal of tops. PARKER and SAMPSON (**14**) report a 15-day cessation of growth in 15-day-old *Bromus hordeaceus* after one clipping. Evidently there is more or less of a balance between the root system and the photosynthetic area necessary to sustain it.

All of the grasses except *Koeleria* produced roots of greater diameter when the tops were not cut. In all cases except *Holcus* the stele of the roots of the clipped plants formed a higher percentage of the root diameter than those of the unclipped. This suggests that the growth of cortex is limited more by scant food supply than is that of stele. In every case the greater number of xylem strands occurred in the larger steles.

Several of the grasses showed marked differences in ability to recover after clipping. *Koeleria* showed no losses, while survival of *Stipa* fell to 65 per cent. in one instance. Under the conditions of the experiment, *Koeleria* was least injured by clipping. This may be due to its slow growth and to the fact that so much of its green tissue is near the soil surface. As measured by reduction in total weight, *Holcus* and *Bromus* were injured most severely. Seedlings are apparently injured to a somewhat greater extent by clipping than are well established grasses. BISWELL (**4**) reports that frequent clipping of grasses grown from sod reduced yield to one-fourth and root weight to one-tenth that of the controls.

Unless it is necessary to conserve soil moisture, as in the case of winter wheat (**5**), grass seedlings should be protected against grazing in order to obtain maximum development of roots and foliage.

Summary

1. Seedlings of six range and pasture grasses were grown in soil in the greenhouse and their development was studied after each of four clipping treatments.

2. Growth of tops, as measured by dry weight, decreased owing to clipping. Reduction in yield ranged from 80 to 96 per cent. for the different species. Elongation of tops was stimulated in one-half the species and inhibited in the other half. Width and number of leaves and number of tillers were reduced by clipping.

3. Removal of tops invariably retarded root penetration but seldom stopped it completely. Nodal roots were the underground parts affected most. In most cases these roots were nearly absent after the fourth clipping, either having failed to develop or having died back from the tips. Excepting in *Koeleria,* the nodal roots of the clipped grasses were of smaller diameter and had relatively larger steles than those of the unclipped plants.

4. The greatest reduction in yield, that of *Bromus,* accompanied the greatest retardation in root development. Minimum reduction in both root and shoot occurred in *Koeleria.* In general, clipping reduced growth of roots, as based on dry weight, about twice as much as that of tops. In *Koeleria,* however, the weight of tops was reduced more than that of roots. In the six species the roots of the clipped plants ranged in weight from one-third to one-fiftieth as much as those of their controls.

5. Excepting in *Koeleria,* the dry weight of roots decreased or remained constant after each clipping. Root volumes correlated fairly well with dry weights.

6. Removal of the aerial parts of grass seedlings had an immediately injurious effect which was measurable both above and below ground. Extent of injury depended largely upon the nature of the species and the frequency of the treatment.

The writer expresses his appreciation to Dr. J. E. Weaver, who suggested the problem and gave much helpful advice throughout the course of the research.

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QUANTITATIVE RELATION BETWEEN CHLOROPHYLL AND IRON IN GREEN AND CHLOROTIC PEAR LEAVES

J. OSERKOWSKY

(WITH SIX FIGURES)

I. Introduction

Lime-induced chlorosis is known to be curable by iron, and for this reason is attributed to iron deficiency. Chlorotic leaves should accordingly contain less iron than green leaves. It has been found by several investigators, however, that chlorotic leaves may contain more iron than green leaves. Similar results were obtained also by the writer, regardless of whether the iron content was reported on the dry or on the fresh-weight basis. No positive correlation could be observed between the iron content and the amount of chlorophyll of leaves obtained from chlorotic trees (table II, columns 4 and 5), although iron was the limiting factor in these leaves in so far as chlorophyll formation was concerned. This fact may be explained by either of the following hypotheses:

1. All the iron present in the leaves is equally (or nearly equally) active in chlorophyll formation. The efficiency of its activity, however, may vary considerably in leaves of the same tree. The green leaves would accordingly be those leaves which may contain small amounts of iron, but in which the efficiency of the iron in chlorophyll formation is very great.

2. Only a fraction of the iron in the leaves is active in chlorophyll formation. This active fraction is more abundant in the green leaves than in the chlorotic ones, although the reverse may be true in the case of the total amount of iron in the leaves.

In regard to the first hypothesis, it may be stated that a wide range of variations in the efficiency of the iron in leaves is logically not impossible. On the other hand, the assumption that all the iron in leaves is active in chlorophyll formation seems improbable, since not all the iron is present in one form. Thus BOUSSINGAULT (**2**) could extract with alcohol only about one-fourth to one-half of the iron content in leaves. SERGER (**11**) also found that not all the iron in spinach leaves could be extracted with alcohol, or with a mixture of benzene, chloroform, and ether. The investigations of SUZUKI (**13**), GRIESSMEYER (**6**), and INGALLS and SHIVE (**7**) indicate also that the iron in leaves is present in more than one form.

This evidence favors the assumption that a specific form of iron is active in chlorophyll formation. This form of iron is designated in this paper *active iron*. In the following pages, the attempts which were made to determine the amount and the nature of this active iron are discussed.

II. Material and methods

Pear trees of two varieties were used, Hardy and Bartlett. The trees grew on soil rich in lime. Most of the samples were taken from two orchards which were badly affected by chlorosis. The trees in these orchards varied greatly in the chlorophyll content of their leaves. It was not uncommon to find individual trees which bore leaves of all shades of color, ranging from cream-yellow to deep green, and often green and chlorotic trees stood side by side.

Repeated treatments over a period of four years showed that the trees in these orchards always responded to application of iron, when applied in any of the following ways: spraying of leaves with iron salt solutions; injection of iron salt solutions into the trunk and limbs; and application of powdered iron salts into holes bored in the lower end of the trunk or branches.[1] Positive results were obtained regardless of the acid radical attached to the iron, provided the iron compounds were fairly soluble. The following compounds were found to induce greening of chlorotic leaves: ferric sulphate, ferric citrate, ferric chloride, ferric oxalate, ferrous sulphate, and ferrous citrate. On the other hand, application of citric acid, tartaric acid, cupric sulphate, manganese sulphate, and magnesium salts failed to give positive results. It is thus obvious that the plants dealt with in this investigation suffered from a typical lime-induced chlorosis due to a deficiency of iron or an abnormal iron metabolism.

Preliminary work had shown that failure to wash the leaves before analysis may vitiate the iron determination by more than 100 per cent. Thus all the leaves were washed well in distilled water before being analyzed. After washing, the leaves were dried at 50°–60° C., and then ground in a porcelain mortar or in a brass mill specially built for the purpose. Care was taken to avoid contact between iron and the samples.

The leaf powder was ashed in porcelain or silica crucibles, and the iron in the ash determined colorimetrically by the thiocyanate method as modified by WALKER (**14**). Care was taken to keep the standard and samples at about the same acidity, namely 0.25 N. The determination of iron in apricot and peach leaves, and some pear leaves, however, was carried out in 1.0 N HCl solutions. Frequent blank determinations were made with porcelain and silica crucibles, and the values for the iron content of the samples were corrected accordingly.

In the presence of small amounts of iron, for example, of 1.0 parts per million or less, in the solution to be analyzed, a modified method was employed similar to that used by STOKES and CAIN (**12**), the method being

[1] The trees were treated by Dr. J. P. BENNETT, and the writer is indebted to him for the use of the data thus obtained. For details concerning the treatments, see BENNETT (**1**).

based on the property of ethyl acetate and amyl alcohol to extract the red iron thiocyanate compound from aqueous solution. To one volume of the acid solution containing iron and ammonium or potassium thiocyanate, one-half to two-thirds' volume of ethyl acetate (or amyl alcohol) was added. The mixture was shaken in a separatory funnel and allowed to stand for a few minutes. The ethyl acetate was then separated from the aqueous solution and the color of the ethyl acetate solution compared with that of a standard solution treated in the same way. It was found that the ethyl acetate intensified the color, made it more stable, and was particularly suitable for the determination of small amounts of iron in the presence of small amounts of copper.

A direct contact was avoided between corks, rubber stoppers, ordinary filter paper, and the acid iron solution, since it was found that these objects may contain sufficient amounts of acid-soluble iron to vitiate the results. The solutions to be analyzed were filtered through acid-washed filter paper, and were kept in glass-stoppered flasks.

Practically all the iron values here reported represent the averages of duplicate or triplicate determinations.

When leaf material was extracted with various solvents, the following procedure was used: to about 4.7–6.6 gm. of dry powdered leaf material in a glass-stoppered flask, 50–70 cc. of solvent were added in proportion to the weight of the sample. The flasks were put in a shaker for about 24 hours.[2] The suspension was then centrifuged for about 20–25 minutes, and to the solid residue about 20–30 cc. of the solvent were added, mixed with a glass rod and centrifuged again for 10–15 minutes, decanted, 20–25 cc. of the solvent added once more, the solution stirred with a glass rod, centrifuged again for 10 minutes, and decanted. The decanted portions from each sample were combined, the liquid evaporated in porcelain or silica crucibles, the residue ashed, and the amount of iron in the ash determined as described.

Chlorophyll was determined always on *fresh leaves* according to the method of WILLSTÄTTER and STOLL (**16,** pp. 2–3). The color of potassium chlorophyllin of the samples was compared with that of a standard solution of potassium chlorophyllin prepared from pure chlorophyll isolated from fresh grass according to the method of WILLSTÄTTER and his co-workers (**16,** pp. 30–32).

[2] While it is not essential to adhere closely to this period of time (24 hours), it is very important when dealing with 1.0 N HCl extraction that the period of shaking should be the same for all samples of a given series, that is, leaves of equal age, collected from the same trees, and on the same date.

III. Active iron in pear leaves

It was believed that in samples of leaves in which active iron was the limiting factor in chlorophyll formation, the amount of green pigments should bear a positive correlation to the amount of active iron they contain. In an attempt to isolate the active iron, green and chlorotic pear leaves of the same age were extracted with various solvents, and the amount of iron in these extracts was compared with the chlorophyll content of the samples to ascertain whether a direct relation existed between them. No such correlation was found when the leaves were extracted with distilled water or with 0.05 N HCl. Similar results were obtained also in regard to the iron in the vacuolar sap of leaves, which was secured by a method similar to that used by CHIBNALL (3): the fresh leaves were washed in water, then dried with a clean towel or filter paper, dipped in ether for 5–10 minutes, spread on filter paper to dry for 10–20 minutes, then pressed in a Buchner press between porcelain or copper plates. The sap obtained in this manner is termed in this paper *vacuolar sap* merely for convenience, since proof is lacking that the liquid obtained is necessarily pure vacuolar sap. The data relating to the extraction of pear leaves with 1.0 N HCl are presented in table I. This table shows that in all samples, with the exception of samples 17 and 18, the amount of iron extracted is higher in the green leaves than in the corresponding chlorotic leaves. This holds true also in the case of samples 13 and 14, in which the total amount of iron present in the green leaves is smaller than that contained in the yellow leaves. Table I thus clearly indicates that a positive correlation exists between the amount of iron extracted from leaves with 1.0 N hydrochloric acid and with their chlorophyll content. It should be emphasized, however, that the two samples (17 and 18) which show exception to this rule were collected late in the season. This fact will be further discussed later.

The data in column 6, however, show also that the total iron in all cases, except samples 13 and 14, is higher in the green leaves than in the corresponding chlorotic ones. This gave rise to the supposition that the amount of iron extracted with 1.0 N hydrochloric acid stood in direct relation to the total iron present in the sample, and that consequently the values presented in column 7 depended on the total iron present, but did not stand in direct correlation to the chlorophyll content of the samples.

In order to test this assumption, a series of pear leaves was collected, and their chlorophyll content, the total amount of iron present, and the amount of iron extracted[3] with 1.0 N hydrochloric acid determined. The

[3] Most of the samples in this series were extracted for 24 hours. While it is not essential to adhere strictly to this period of shaking, it is very important that samples belonging to the same series (*e.g.*, collected from the same trees at a given date) should be extracted for the same length of time.

TABLE I

TOTAL AMOUNT OF IRON IN PEAR LEAVES AND AMOUNT OF IRON EXTRACTED FROM THEM WITH 1.0 N HCl

No. of sample	Condition of leaves	Description of sample	Variety	Date collected	Total iron in leaves	Iron in 1.0 N HCl extract
					In p.p.m. of dry weight of leaves	
1	Green	Spur leaves from one-year-old wood	Bartlett	Apr. 20/29	70	26
2	Chlorotic			"	42	16
3	Green			May 16/29	100	39
4	Chlorotic			"	97	23
5	Green			Aug. 15/29	117	60
6	Chlorotic			"	73	29
7	Green	Leaves from base of shoots	Bartlett	May 29/29	98	47
8	Chlorotic			"	63	27
9	Green	Leaves from middle of shoots	Bartlett	Aug. 17/29	92	47
10	Chlorotic			"	69	32
11	Green	Spur leaves from wood older than one year	Hardy	Apr. 17/29	42	20
12	Chlorotic			"	25	8
13	Green			May 28/29	49	23
14	Chlorotic			"	70	16
15	Green			Aug. 5/29	79	42
16	Chlorotic			"	76	26
17	Green			Aug. 16/27	76	33
18	Chlorotic			"	120	41

sampling was done as follows: severely chlorotic, moderately chlorotic, and green leaves of the same age were collected from the same chlorotic trees. The samples designated as "green, treated with iron in 1928" were obtained from chlorotic trees which were treated with iron in December, 1928, and in consequence of which bore very green leaves in the 1929 and 1930 seasons.

The results of the analyses are presented in table II, columns 6 and 7, from which it is concluded that no correlation exists between the total iron content of these samples and the quantity of iron which is extracted from them with 1.0 N hydrochloric acid. On the other hand, this table reveals

TABLE II

ACTIVE IRON, CHLOROPHYLL CONTENT, AND AMOUNT OF IRON EXTRACTED FROM HARDY PEAR LEAVES WITH 1.0 N HCl

No. of sample	Description of leaves	Leaves taken from	Date of collecting sample	Chlorophyll content in % of dry weight of leaves	Total iron in leaves	Total iron in 1.0 N HCl extract	Inactive iron in 1.0 N HCl extract	Active iron
					p.p.m. of dry weight of leaves			
1	Severely chlorotic	Spurs	April 29	0.084	33	14.6	13.2	2.4
2	Moderately chlorotic	Spurs	"	0.18	40	19.4	13.2	5.1
3	Green, from chlorotic trees	Spurs	"	0.53	33	27.1	13.2	15.0
4	Severely chlorotic	Spurs	May 13	0.11	37	9.2	6.4	2.7
5	Moderately chlorotic	Spurs	"	0.18	27	10.7	6.4	4.5
6	Green, from chlorotic trees	Spurs	"	0.28	36	12.8	6.4	7.1
7	Green, from trees treated in 1928	Spurs	"	0.65	51	23.2	6.4	16.2
8	Severely chlorotic	Spurs	May 27	0.073	32	12.0	10.0	1.2
9	Moderately chlorotic	Spurs	"	0.15	29	12.0	10.0	2.6
10	Green, from chlorotic trees	Spurs	"	0.34	32	16.4	10.0	5.9
11	Green, from trees treated in 1928	Spurs	"	0.77	54	22.5	10.0	13.3
12	Severely chlorotic	Spurs	June 16	0.061	49	14.9	12.3	1.0
13	Moderately chlorotic	Spurs	"	0.18	47	15.9	12.3	2.9
14	Green, from chlorotic trees	Spurs	"	0.42	48	17.5	12.3	6.9
15	Green, from trees treated in 1928	Spurs	"	0.93	63	27.3	12.3	15.2
16	Severely chlorotic	Spurs	July 9	0.058	62	15.8	14.7	1.4
17	Moderately chlorotic	Spurs	"	0.18	63	18.9	14.7	3.6
18	Light green from chlorotic trees	Spurs	"	0.31	64	22.1	14.7	7.2
19	Deep green from chlorotic trees	Spurs	"	0.44	78	26.1	14.7	10.8
20	Severely chlorotic	Base of shoots	"	0.071	76	22.3	19.1	2.0
21	Moderately chlorotic	Base of shoots	"	0.22	66	24.0	19.1	6.0
22	Light green from chlorotic trees	Base of shoots	"	0.29	.....	26.4	19.1	8.0
23	Deep green from chlorotic trees	Base of shoots	"	0.68	74	37.4	19.1	18.3
24	Severely chlorotic	Middle of shoots	"	0.093	.....	21.1	17.7	1.8
25	Moderately chlorotic	Middle of shoots	"	0.20	48	19.6	17.7	3.8
26	Deep green from chlorotic trees	Middle of shoots	"	0.65	57	29.9	17.7	12.2
27	Severely chlorotic	Terminal of shoots	"	0.063	59	17.8	16.7	1.0
28	Moderately chlorotic	Terminal of shoots	"	0.086	54	17.8	16.7	1.2
29	Deep green from chlorotic trees	Terminal of shoots	"	0.60	62	25.0	16.7	8.3

a striking *positive correlation between the amount of iron extracted from the leaves and their chlorophyll content* (see figure 1).

This fact strongly suggests that the active iron, or its hydrolysis product, is contained in the 1.0 N hydrochloric extract. It remains to be determined whether this extract contains *only* the active iron, or whether it contains in addition some other fractions of iron which are inactive.[4]

Since iron in these samples is the limiting factor in so far as chlorophyll formation is concerned, it was reasoned that if all the iron in the extract were active iron, then a more or less direct proportionality should exist between the amount of chlorophyll in samples of each series and the total amount of iron extracted from them by 1.0 N hydrochloric acid. On the other hand, if the 1.0 N hydrochloric extract contained also inactive iron, then such a proportionality should exist only between the active iron fraction and the chlorophyll content. The data of table II are plotted in figure 1, where the chlorophyll content is represented by the ordinate, and the total amount of iron extracted from the leaves with 1.0 N hydrochloric acid is plotted on the abscissa. This figure shows that no direct proportion exists between the *total amount* of extracted iron and the chlorophyll content; hence it is concluded that the 1.0 N hydrochloric acid extract contains some inactive iron in addition to the active iron.

It could have been argued that the iron in the 1.0 N hydrochloric acid extract was active iron only, but that this extract contained merely part of the total active iron present in the leaves. If this were true, then sample 14 (table II), for example, which contains about 6.9 times more chlorophyll than sample 12, should contain also about 6.9 times more active iron than sample 12; in other words, it must contain at least $6.9 \times 14.9 = 102$ p.p.m., which is greatly in excess of the total iron present in sample 14, namely 48 p.p.m.

Figure 1 also indicates that all samples in each series (*e.g.*, samples of the same age, collected from the same trees, and on the same date) lie on a straight line, or their distance from a straight line drawn through them is within the experimental error involved in the determination of their iron content. For most samples in table II the difference between duplicate iron determinations of 1.0 N hydrochloric extracts lies within 5 per cent. of the average value. The error involved in the chlorophyll determination for the values given in this table may therefore amount to as much as 10–15 per cent. This is due in part, presumably, to the variation in chlorophyll content within leaf material of a given sample.

[4] The terms *active iron* and *inactive iron* used in this paper refer to the iron fractions which are active or inactive in the formation of chlorophyll only. These terms do not imply anything in regard to the activity of these fractions in other physiological processes, *e.g.*, respiration.

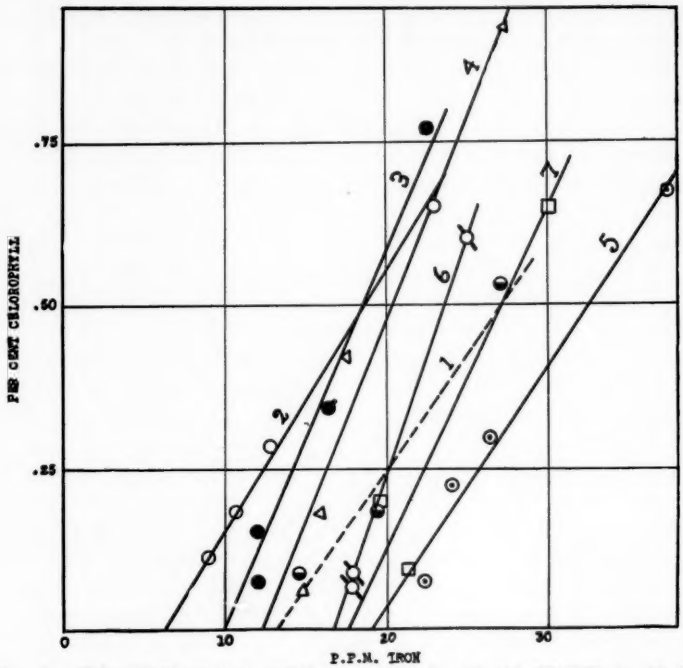

FIG. 1. Chlorophyll content and iron extracted with 1.0 N HCl in 24 hours: 1, ◒ = spur leaves collected April 29; 2, ○ = spur leaves collected May 13; 3, ● = spur leaves collected May 27; 4, △ = spur leaves collected June 16; 5, ⊙ = leaves from base of shoots collected July 8; 6, ◯ = leaves from terminal shoots collected July 9; 7, □ = leaves from middle of shoots collected July 7.

This fact may be readily explained on the assumption that *all 1.0 N hydrochloric acid extracts of samples belonging to the same series contain the same amount of inactive iron, and differ only in the amount of active iron they contain.* (The validity of this statement will be discussed later on, and it will be shown that it does not always hold true. This, however, does not affect the conclusions drawn here, since this assumption holds true for the samples given in figures 1 and 5.) Thus the difference in the amount of extracted iron of two samples belonging to the same series corresponds to the difference in the amount of active iron.

If, in figure 1, a straight line be passed through points belonging to samples of one series, then the point of intersection of this line with the abscissa will correspond to a hypothetical sample of chlorotic leaves of which the chlorophyll content is equal to 0. It is evident that the amount of active iron in such a sample must be extremely small, or equal to 0. It

then follows that the distance between this intersection point and the origin represents the amount of the *inactive iron* which a 1.0 N hydrochloric extract of such a sample would contain. But this amount of inactive iron represents, according to our assumption, the amount of inactive iron in the extracts of all other samples of this series; hence the amount of active iron in a sample of this series is given by the expression: $Fe_a = Fe - Fe_i$, where Fe_a is the active iron; Fe_i is the inactive iron in the 1.0 N HCl extract; and Fe represents the total iron in the same extract.

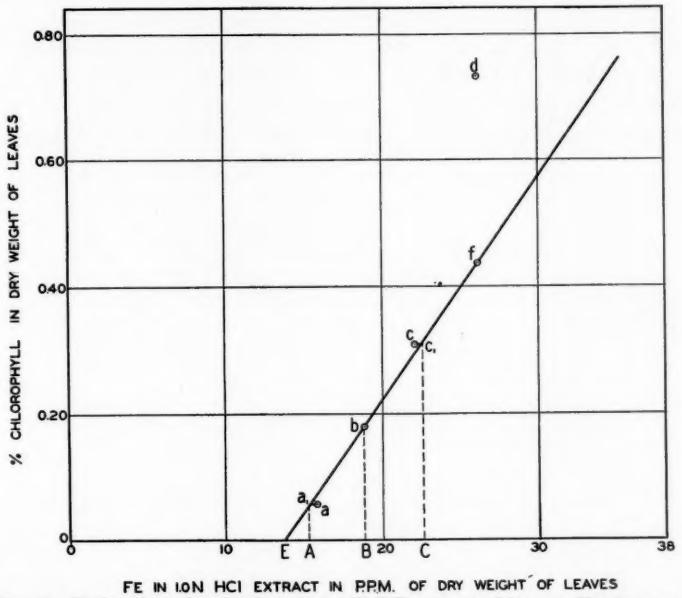

FIG. 2. Estimation of active iron in 1.0 N HCl extract of leaves; Hardy spur leaves collected July 9, 1930.

An example will serve to illustrate the method of estimating the active iron in pear leaves. In figure 2 the points *a*, *b*, *c*, and *f* represent severely chlorotic, moderately chlorotic, slightly green, and very green leaves from chlorotic trees respectively, collected on July 9, 1930. These samples were spur leaves collected from the same trees, and therefore belong to the same series; while sample *d* was collected at the same time and in the same orchard, but from a different group of trees which were treated with iron in 1928. The line $a_1 f$ is the straight line of closest fit to pass between the points *a*, *b*, *c*, and *f*. This line intersects the abscissa at the point *E*. Thus *OE* represents the inactive iron for this series of samples. The active iron for

sample a is obtained by drawing from a a line parallel to the abscissa; this line intersects $a_1 f$ at a_1. From a_1 a perpendicular line to the abscissa is drawn which intersects it at A; EA is thus the active iron for a, while EB and EC represent the active iron for b and c respectively. The values of Fe for several series of Hardy pear leaves are plotted in figure 1 against the chlorophyll content, and straight lines are drawn through points belonging to the same series, which allow the estimation of the active iron. The values thus obtained are presented in table II, column 9.

In figure 3 the values of Fe_a are plotted on the ordinate against the value of $(Fe - Fe_l)$ on the abscissa on the same scale. The points on figure 3 represent 29 samples comprising 8 series and collected at different times of the season between April and July. These samples include spur leaves as well as shoot leaves,[5] all of which were collected from Hardy trees in a chlorotic orchard. If Fe_a were equal to $(Fe - Fe_l)$, then all the points

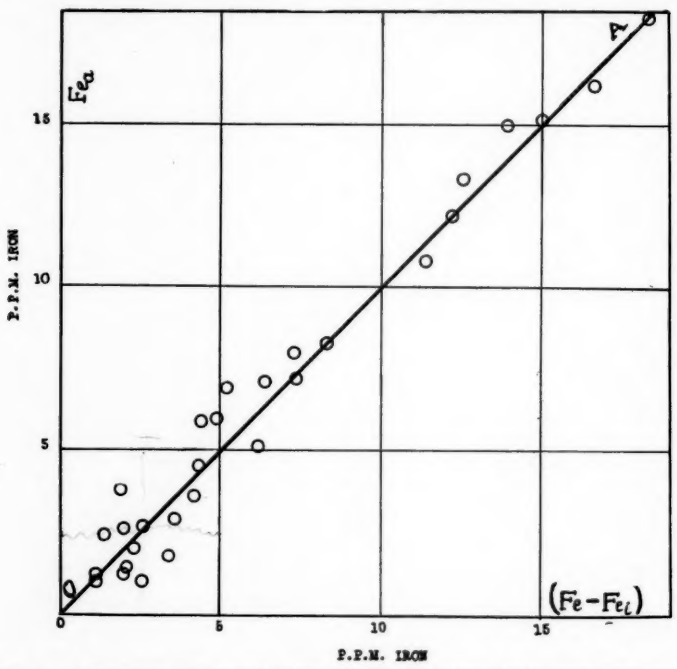

FIG. 3. Active iron (Fe_a) and values of ($Fe-Fe_l$) for Hardy pear leaves.

[5] In the case of shoot leaves, each series comprised leaves of nearly the same age. This was accomplished by segregating the leaves from the base, middle, and top of the shoots to make separate samples.

in figure 3 should lie on a straight line, OA, passing through the origin and making an angle of 45° with either one of the axes. The positions of the points on figure 3 afford a means of gauging the agreement between the calculated values of the active iron (that is, Fe_a) and the observed values. This figure shows that out of 29 samples there is not a single one for which the value of $\triangle$ Fe, as defined by the expression $\triangle Fe = Fe_a - (Fe - Fe_l)$, equals or exceeds 2 p.p.m., and for 22 samples $\triangle$ Fe is less than 1 p.p.m. This is very good agreement, since the values of $\triangle$ Fe are well within the limit of the error involved in the determination of Fe.

The data plotted in figures 1 and 2 pertain only to the samples which were collected up to July 9; samples collected later do not show a consistent correlation between the amount of iron extracted in 24 hours with 1.0 N HCl and their chlorophyll content, as can be seen from table III and from figure 4 which represents the results obtained for Hardy samples collected on August 7. This finding is in agreement with the data reported in table I (samples 17 and 18), in which it was shown that the 1.0 N HCl

TABLE III

CHLOROPHYLL CONTENT, AND THE IRON EXTRACTED WITH 1.0 N HCl FROM PEAR LEAVES COLLECTED FROM CHLOROTIC TREES LATE IN GROWING SEASON

DESCRIPTION OF LEAVES	VARIETY	ORCHARD	DATE OF COLLECTING SAMPLES	CHLOROPHYLL CONTENT IN % OF DRY WEIGHT OF LEAVES	TOTAL IRON EXTRACTED WITH 1.0 N HCl IN P.P.M. OF DRY WEIGHT OF LEAVES
Severely chlorotic	Bartlett	Mc	July 20	0.087	53.3
Moderately chlorotic	"	"	"	0.27	43.3
Light green	"	"	"	0.42	39.5
Deep green	"	"	"	0.68	54.1
Severely chlorotic	Hardy	M	"	0.10	16.4
Moderately chlorotic	"	"	"	0.26	25.0
Light green	"	"	"	0.43	22.5
Deep green	"	"	"	0.96	36.1
Severely chlorotic	"	"	August 7	0.056	28.1
Moderately chlorotic	"	"	"	0.20	22.0
Light green	"	"	"	0.25	30.0
Deep green	"	"	"	0.66	34.2
Severely chlorotic	"	B	"	0.045	43.1
Moderately chlorotic	"	"	"	0.14	38.5
Light green	"	"	"	0.38	40.4
Deep green	"	"	"	0.73	54.8

extract of a chlorotic sample collected in August contains more iron than a similar extract from green leaves.

An explanation for this fact may be furnished by the observations of SACHS (**10**) and other workers. SACHS noticed that chlorotic leaves did not respond to treatment of iron when applied late in the season. ZIMMERMAN (**17**) and ROUX (**9**) reported that the chloroplasts of severely chlorotic leaves showed marked signs of injury, which finally resulted in the disintegration of the plastids. These observations indicate that some profound changes take place in chlorotic leaves late in the season, which may affect the solubility of the various iron compounds of the leaves. It is likely that the hydrochloric acid extract of chlorotic samples collected late in the season may contain amounts of inactive iron different from those of the extracts from green leaves. The active iron cannot be determined in samples collected late in the season, since the method of its estimation, previously described, can be used only when all the samples of a series contain in their hydrochloric acid extract the same amount of inactive iron.

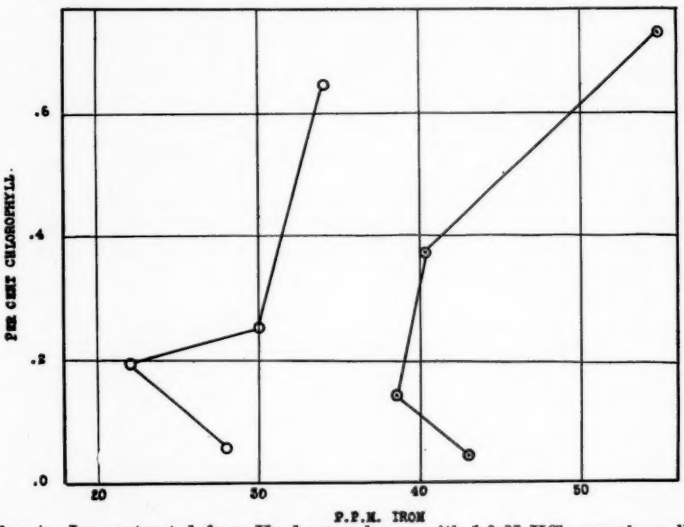

FIG. 4. Iron extracted from Hardy spur leaves with 1.0 N HCl; samples collected August 7: ——○——, leaves from orchard M; ——⊙——, leaves from orchard B.

Again, the injury to the leaf caused by prolonged chlorosis may impair the efficiency of the active iron, until the chloroplasts are injured beyond recovery, when the active iron, even if abundant, may fail to bring about the formation of chlorophyll. If this were the case, then the amount of

active iron in leaves of the same age, collected from the same trees, would not be proportional to their chlorophyll content.

From the foregoing it is obvious that the method of determining the active iron is limited in its application for the following reasons:

1. In order to estimate the active iron in any one sample, it is necessary to determine the chlorophyll content and the acid-extractable iron of a whole *series* of samples (the series should consist of at least three samples).

2. The leaf samples in each series must be of the same age and grown on the same trees; they must, however, differ markedly in their chlorophyll content.

3. The active iron must be the limiting factor in each sample of a series, in so far as chlorophyll formation is concerned.

4. The method cannot be applied to samples collected late in the season (*e.g.*, August or later).

On account of these limitations, a method which could be of wider application is being investigated at the present time.

IV. Active iron in peach and apricot leaves

Green and chlorotic peach and apricot leaves were collected from chlorotic trees. The chlorophyll content, total amount of iron, and iron extracted from the leaves with 1.0 N HCl were determined by the same procedure as used with pear leaves. The data obtained are presented in table IV and figure 5. It may be noted that while the total amount of iron in the chlorotic leaves is smaller than that present in the green leaves,

TABLE IV

ACTIVE IRON IN PEACH AND APRICOT LEAVES FROM CHLOROTIC TREES. SAMPLES COLLECTED JULY 20 FROM MIDDLE OF SHOOTS

DESCRIPTION OF LEAVES	CHLOROPHYLL CONTENT IN % OF DRY WEIGHT	TOTAL IRON IN LEAVES	IRON IN 1.0 N HCl EXTRACT	ACTIVE IRON
		IN P.P.M. OF DRY WEIGHT OF LEAVES		
Severely chlorotic peach leaves	0.21	41	14.0	3.8
Moderately chlorotic peach leaves	0.68	48	22.3	10.1
Light green peach leaves	1.09	75	30.2	19.6
Deep green peach leaves	1.47	75	36.9	26.7
Severely chlorotic apricot leaves	0.16	48	15.5	5.1
Moderately chlorotic apricot leaves	0.31	45	18.0	9.4
Light green apricot leaves	0.59	76	30.8	17.8
Deep green apricot leaves	0.90	62	37.7	27.2

no consistent relation exists between the total iron content and the chlorophyll content, while the relation between the amount of iron extracted with 1.0 N HCl and the chlorophyll content is very close. It is evident that the method of estimation of active iron as described in this paper is applicable to leaves of pear, apricot, and peach, and presumably also to other material.

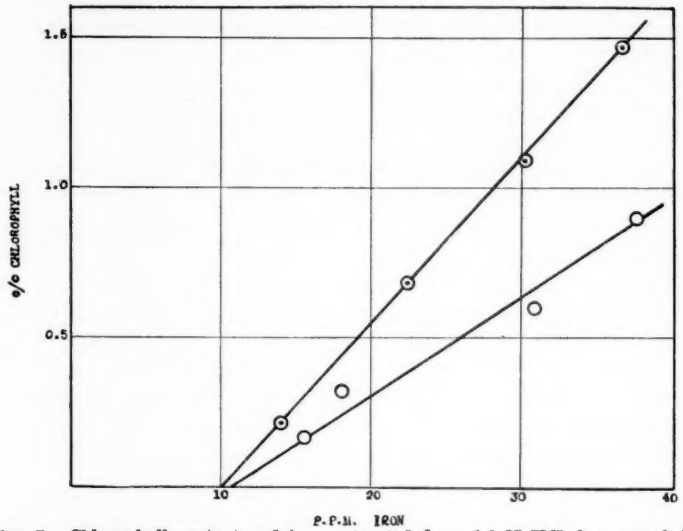

FIG. 5. Chlorophyll content and iron extracted from 1.0 N HCl from peach leaves (⊙) and apricot leaves (○).

V. Nature of active and inactive iron

When a solution of ammonium thiocyanate or potassium thiocyanate is added to 1.0 N HCl extract of pear leaves, the brown liquid turns red-brown. This indicates the presence of Fe^{+++} ions, or of an iron compound readily converted into Fe^{+++}. The 1.0 N HCl extract is deeply colored, however, owing to the presence of decomposition products of chlorophyll and of other compounds. For this reason the intensity of the red color of the iron thiocyanate cannot be determined directly on the extract. In order to separate the ionic iron from the rest of the extract, the following procedure was adopted.

The 1.0 N HCl extract of a leaf sample was made up to 175 cc. with 1.0 N HCl solution, and 25 cc. of 40 per cent. NH_4CNS were added to it. To this solution 50 cc. of ethyl acetate were added; the whole was shaken for a minute or two in a separatory funnel, the emulsion allowed to stand for 10–20 minutes, the aqueous phase drained, and the ethyl acetate collected

in a flask. The extraction with ethyl acetate was repeated five or six times until the ethyl acetate remained colorless. The ethyl acetate extracts were added together and evaporated slowly in pyrex beakers on a hot plate at a low heat. When the volumes of the liquid in the beakers were reduced to about 10–20 cc., the beakers were removed from the hot plates and allowed to cool, and concentrated nitric acid was added drop by drop, a few drops at a time with an interval of several minutes between each addition. After this process was repeated several times, about 5 cc. of concentrated nitric acid were added and the liquid in the beakers was evaporated at low heat until dry. The beakers were then put in an electric furnace and the residue ashed at low temperature (at a very dull red). The determination of the iron in the ash was carried on in the manner previously described.

TABLE V

IONIC AND IONIZABLE IRON IN 1.0 N HCl EXTRACTED FROM PEAR LEAVES

DESCRIPTION OF SAMPLE	DATE OF COLLECTING SAMPLE	HOURS OF EXTRACTION WITH 1.0 N HCl	TOTAL IRON IN 1.0 N HCl EXTRACT	IRON IN ETHYL ACETATE EXTRACT	IRON IN ACID RESIDUE	Fe_a
			IN P.P.M. OF DRY WEIGHT			
Severely chlorotic spur leaves	July 9	44	12.8	8.3	4.5	1.4
Light green spur leaves	"	47	24.7	21.2	3.5	7.2
Light green leaves from shoot terminals	"	7	15.2	12.7	2.5	8.3

The results of the ethyl acetate extractions are presented in table V. Practically all the iron in the HCl extract was removed by ethyl acetate. The small quantities found in the acid residue may have been due partly to traces of iron in the reagents used, and partly to some ethyl acetate which remained as a fine emulsion in the acid phase. The data in table V indicate that *practically all of the iron in the acid extract is present as ferric iron, or in a form which is readily converted into* Fe^{+++}. In this respect no difference exists between the active and the inactive iron in 1.0 N HCl.

This fact, however, does not disclose in what form the active and the inactive iron respectively are present in the living cell. So much, however, can be concluded: *these two forms of iron are present in the leaf cells in compounds which can be dissolved, or readily converted by 1.0 N HCl into ionic iron or ionizable iron.* It may be of interest to note that all the iron

compounds used successfully in this investigation for the cure of chlorosis were compounds which in aqueous solutions dissociate, at least partly, into ferric or ferrous ions. An attempt to treat chlorotic trees with potassium ferrocyanide, which in aqueous solution does not yield ionic iron in appreciable amounts, was not successful on account of the injurious effects of potassium ferrocyanide on the trees. No conclusion, therefore, can be drawn from this experiment regarding the ability of pear leaves to convert a non-ionic iron compound into active iron.

Since it has been found in several leaf samples that the amount of active iron greatly exceeds the amount of iron extracted with water, or the amount of iron contained in the "vacuolar sap," it is thus inferred that *the active iron is not present in these leaf samples in solution; at the most only part of it is soluble.*

Pear leaf tissue tested microchemically for iron with potassium ferrocyanide and potassium ferricyanide yielded negative results. Positive tests were obtained only with leaves which were taken from trees treated with iron. The positive reaction in these leaves was observed only in and near parts which showed injury effects due to an excess of iron. The microchemical tests thus carried out were not numerous, but the results obtained are in agreement with those of MILAD (8). This is not necessarily proof that no ionic iron readily soluble in hydrochloric acid exists in pear leaves. It has already been shown that the amount of active iron in pear leaves is not large, and it probably seldom exceeds 50–80 p.p.m. of the dry weight; often it is much less than that. Such an amount of iron when distributed in an excess of reagent may be diluted to the extent that it remains in solution notwithstanding the presence of large amounts of ferri- or ferrocyanide.

TABLE VI

VALUES OF E FOR VARIOUS LEAF SAMPLES

LEAVES COLLECTED FROM	PLANT	DATE OF COLLECTING SAMPLE	E
Spurs	Hardy pear	April 29	22.1
Spurs	"	May 13	25.1
Spurs	"	May 27	37.7
Spurs	"	June 16	38.3
Spurs	"	July 9	22.0
Base of shoots	"	July 9	22.7
Middle of shoots	"	July 9	33.2
Terminal end of shoots	"	July 9	44.3
Middle of shoots	Peach	July 20	33.4
Middle of shoots	Apricot	July 20	20.7

It is also possible that an appreciable fraction of the ionic iron is present as complex in the HCl extract, tied up with organic acids. The iron in these complexes may be readily ionizable, but the concentration of F^{++} or Fe^{+++} in the extract may be too small to yield a positive test with $K_3Fe(CN)_6$ or $K_4Fe(CN)_6$.

The slope of the lines in figure 1 and figure 5 is given by the ratio $E = \frac{\text{chlorophyll content}}{\text{active iron}}$ per unit weight of leaves. This ratio is therefore a measure of the efficiency of the active iron in chlorophyll formation. In table VI the values of E are presented for ten series of samples. The values of E in this table are expressed in mols of chlorophyll[6] per gram atom of active iron. No consistent correlation is revealed between the age of the leaves and the value of E. The fact that the values of E are large and variable leads to the conclusion that it is *highly improbable that the active iron is combined with the chlorophyll in a stoichiometrical relation* if it is combined with it at all.

Iron is known to catalyze oxidation processes in living substances. The formation of chlorophyll in the living plants is, most likely, associated with an oxidation process, since several investigators claimed that the rate of chlorophyll formation in seedlings was greatly impeded by low oxygen pressure in the air surrounding the plants (for example, CORRENS **4**). The active iron presumably does not form a part of the chlorophyll molecule; it is likely, therefore, that its function consists in catalyzing an oxidation process or some oxidation processes connected with chlorophyll formation.

EMERSON (**5**) succeeded in growing *Chlorella* in sugar solutions deficient in iron. The algae in such solutions were devoid of, or deficient in, chlorophyll; but their rate of respiration was substantially the same as that of normally green *Chlorella.* The fact that the chlorotic algae had a normal respiratory rate suggests that they were not subnormal in regard to the amount of "respiratory ferment" they contained. In spite of this they were decidedly subnormal in their chlorophyll content. It seems, therefore, improbable that the active iron is identical with the respiratory iron ferment of WARBURG (**15**).

The nature and the localization in the cell of the inactive iron are also uncertain. The inactive iron extracted with 1.0 N HCl may differ from the active iron only in regard to its localization in the cell, *i.e.*, it may be present only in the interior of plastids or other protoplasmic bodies. On the other hand, it is equally plausible that the inactive iron compound may differ from the active in its chemical composition; nor is it certain that the

[6] One mol of chlorophyll was taken as equal to 897.4 which represents an average value for chlorophyll ($a+b$), on the assumption that the ratio of chlorophyll a to chlorophyll b is 3.

inactive iron in the acid extract is derived from one compound only. This last statement may be applied also to the active iron.

The writer wishes to emphasize that while it is common to find chlorotic leaves which contain as much or more iron than green leaves of the same age, the inability to utilize iron for a normal development of chlorophyll is confined to leaves with a low content of iron during at least the first part of the growing season. This statement does not necessarily apply, of course, to yellow leaves in which the development of chlorophyll is abnormal, due to other causes than those which are responsible for lime-induced chlorosis.

This fact would indicate that the occurrence of chlorosis is not entirely independent of the amount of iron in the leaves. Indeed, a comparison of the iron content of leaves in the chlorotic orchard C with that of green leaves from orchard S, in a region free of chlorosis (fig. 6), shows that the

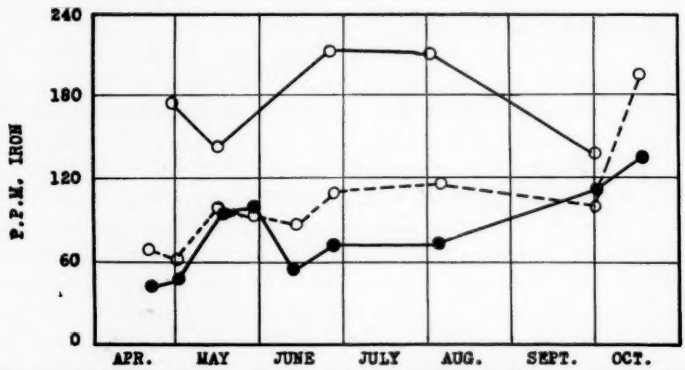

FIG. 6. Seasonal variation of iron content (on dry-weight basis) in Bartlett spur leaves of one-year-old wood: ——●——, chlorotic leaves, orchard C; ---○---, green leaves, orchard C; ——○——, green leaves, orchard S.

iron in leaves from orchard S is on a higher level *throughout* the period of active growth. From experience the writer is inclined to conclude that the occurrence of chlorosis is highly improbable in pear leaves, the iron content of which remains above, say, 70–80 p.p.m. (on the dry-weight basis) during the first two or three months of their growth. An explanation for this fact is offered by the suggestion that a certain equilibrium exists between the active iron and the inactive iron in pear leaves. In leaves containing a small amount of iron, the balance between the two forms of iron may be shifted in such a way as to prevent the formation of an adequate amount of active iron for normal chlorophyll formation; while in leaves rich in iron, the active iron (although it may be only a small fraction of the total

iron) is present in sufficient amount for the normal development of chlorophyll.

Summary

1. Chlorotic pear leaves may contain as much or more iron than green leaves of the same age and taken from the same trees, regardless of whether the iron content is expressed on the fresh-weight or the dry-weight basis. The iron content, however, of green leaves from trees grown in districts free from chlorosis is higher than the iron content of either green or yellow leaves from chlorotic trees. Lime-induced chlorosis (dealt with in this paper) is confined to leaves in which the iron content is relatively low during the first two or three months of the growing season.

2. No correlation exists between the amount of iron extracted from pear leaves with water and with 0.5 N HCl and the chlorophyll content of leaves.

3. Only part of the iron in leaves, the *active iron,* is effective in chlorophyll formation.

4. A method is described for the estimation of the active iron in leaves, which is based on the assumption that the active iron, or its derivative, is contained in the 1.0 N HCl extract of dried leaves.

5. The chlorophyll content of leaves from chlorotic plants is proportional to the amount of active iron in the leaves.

6. The iron of the compound active in chlorophyll formation is present in the 1.0 N HCl extract as ionic iron or in a compound which readily yields ionic iron.

7. The values of the ratio $\frac{\text{chlorophyll}}{\text{active iron}}$ in leaves varies widely in different sets of leaves. It is inferred from this fact that the active iron is not present in leaves in a stoichiometrical combination with chlorophyll, if it is combined with it at all.

The writer wishes to express his appreciation to Dr. J. P. BENNETT for the valuable assistance, suggestions, and criticisms offered throughout the work.

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INDIRECT COLORIMETRIC METHOD FOR THE DETERMINATION OF CALCIUM[1]

E. M. EMMERT

Introduction

The method described in this paper enables the rapid and accurate determination of calcium in a series of samples which do not differ greatly in their calcium content. As with other colorimetric methods, it is particularly useful when small amounts of the element are to be determined. It differs from other colorimetric methods in that large amounts of Ca can also be determined accurately by proper adjustment of reagents.

When the amount of calcium is relatively large and large differences from sample to sample are to be expected, the oxalate titrimetric method of MCCRUDDEN (5) would be preferred, since under these conditions numerous preliminary tests would be necessary in the colorimetric method here presented. This colorimetric method, however, is especially well adapted to detect the effect of varying treatments on the calcium content of biological material in which these treatments cause only small but significant variations in the calcium content. In this case few preliminary tests would be necessary. If phosphorus is to be determined at the same time as calcium, the colorimetric method enhances rapidity as well as increases accuracy for small amounts of calcium.

Basic principles

When excess sodium hydroxide is added to a solution containing iron, magnesium, calcium, and phosphate, in the absence of ammonia salts, iron and magnesium are precipitated as hydroxides while calcium is precipitated as tricalcium phosphate. Iron and magnesium phosphates are not precipitated because they are more soluble than the hydroxides of these metals. Calcium hydroxide, however, is relatively soluble as compared with tricalcium phosphate; therefore only calcium causes a reduction in the phosphate content of the alkaline solution. This reduction of phosphate may be measured very accurately by the colorimetric method for phosphorus, and from this the amount of calcium may be calculated.

Interference

Significant amounts of the ammonium ion would interfere since magnesium hydroxide would tend to be dissolved and magnesium ammonium

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with permission of the Director.

phosphate precipitated. The ammonium ion may be eliminated in the usual ways, such as by igniting the salts or boiling with strong sodium hydroxide.

Any material which colors the solution and is not precipitated out of the solution by sodium hydroxide will interfere. If the color is organic it may be oxidized and eliminated by sodium chlorate (**1**) or ignition in a muffle furnace. Aluminum and most other metals likely will act similarly to iron and not interfere, although if some such elements are present in significant amounts their effect on phosphate in alkaline solution should be tested.

If the amount of phosphate in the sample is large as compared with the calcium present, the method is not very accurate, since the reduction in color by the calcium will be too small. This may be overcome by adding a known amount of calcium to reduce the phosphate color to the correct point and computing the calcium by subtracting the added calcium from the total calcium found.

Determination of phosphate

The exact amount of phosphate in the unknown must be found preliminary to the calcium determination. Take a suitable neutral aliquot (10–70 cc.), make to about 70 cc., add 10 cc. of 2.5 per cent. ammonium molybdate in 5 N sulphuric acid and 3 cc. of 1, 2, 4 amino-naphthylsulphonic acid (**4**), and make up to 100 cc. After five minutes compare with a standard blue phosphate solution which was prepared at the same time as the unknown. This is made by developing the color from 1 cc. of the dilute phosphate standard (reagent 3) as just described.

Approximate determination of calcium

To a 20-cc. aliquot of the calcium unknown solution add enough phosphate to make about 0.3 mg. of P with the phosphate already in the unknown. Make alkaline to phenolphthalein with 10 per cent. sodium hydroxide and add 2 cc. in excess. Make up to 50 cc., shake, and filter through a dry filter. Test a 20-cc. aliquot for phosphate. If no blue color appears, more than 0.6 mg. of calcium is present. To another 20 cc. of the filtrate add enough phosphate standard to make about 3 mg. of P. Shake intermittently for a few minutes and filter. If no blue color appears, more than 6 mg. of calcium are present. Repeat with varying amounts of phosphate until a rough approximation of the calcium is obtained, judging the amounts from the colors developed.

Accurate determination of calcium

The exact quantity of phosphate in the unknown solution and the approximate range of calcium must have been determined as directed previously. For a series of samples of nearly the same calcium content one approximate determination of calcium is sufficient.

To an appropriate aliquot of the calcium unknown add sufficient standard phosphate so that, with what is already in the solution, the total amount is brought up to about the amount indicated in table I for the approximate range of calcium as already found in the sample. The exact phosphate present must be known and should be sufficient so that the calcium present will precipitate between one-fourth and three-fourths of the phosphate present. Add a few drops of phenolphthalein and calcium-free 10 per cent. sodium hydroxide solution until a pink color persists. Add 2 cc. of the sodium hydroxide in excess, shake intermittently for about a minute, and make to 50 cc. Shake a few seconds and filter through a dry no. 2 Whatman filter or through any filter as retentive as this type of paper. Take an aliquot such that the phosphate present is in the range of about 0.1 to 1.0 mg. of phosphate phosphorus. Add enough dilute sulphuric acid just to dispel the pink phenolphthalein color. Determine the phosphate phosphorus left as directed for determining the phosphate in the sample. From the quantity of phosphate in the solution before making it alkaline, and that left after making it alkaline and filtering, the exact amount of calcium may be calculated.

TABLE I

AMOUNTS OF PHOSPHATE STANDARD TO BE USED

APPROXIMATE MG. OF CA	AMOUNT OF P NEEDED	P REAGENT	CA MILLIGRAM EQUIVALENTS OF P SOLUTION
mg.	*mg.*	*cc.*	*mg.*
0.1– 0.5	0.3099	1 (dilute)	0.6
0.5– 1.0	0.6198	2 "	1.2
1.0– 5.0	3.0986	10 "	6.0
5.0–10.0	6.1972	20 "	12.0
10.0–20.0	12.3944	3 (concentrated)	24.0
20.0–30.0	18.5916	6 "	36.0
30.0–40.0	24.7888	8 "	48.0

Reagents

1. Concentrated standard phosphate solution: dissolve 3.398 gm. KH_2PO_4 in distilled water and make to 250 cc.; 1 cc. equals 3.0966 mg. of P, which is equivalent to 6 mg. of calcium.

2. Dilute standard phosphate solution: dilute 10 cc. of reagent 1 to 100 cc.

3. Standard calcium solution: dissolve 0.2498 gm. $CaCO_3$ in distilled water and just enough HCl, and make to 250 cc.; 1 cc. equals 0.4 mg. of Ca.

Results

Table II shows the results of calcium determinations made in the presence of iron and magnesium.

TABLE II

DETERMINATION OF CALCIUM IN PRESENCE OF IRON AND MAGNESIUM

Fe present	Mg present	Ca added	Ca found	Percentage error
mg.	*mg.*	*mg.*	*mg.*	%
1.0	0.4	0.1	0.1	0.0
0.4	0.4	0.4	0.400	0.0
0.4	0.4	0.4	0.399	−0.25
0.4	0.4	0.4	0.397	−0.75
0.4	0.2	1.6	1.590	−0.60
4.0	2.0	2.0	2.050	2.50
4.0	2.0	2.0	2.050	2.50
8.0	4.0	2.0	2.003	0.15
4.0	2.0	4.0	4.000	0.0
8.0	4.0	4.0	3.981	−0.47
8.0	4.0	4.0	4.098	2.45
8.0	4.0	4.0	3.981	−0.47
8.0	4.0	8.0	7.930	−0.88
4.0	4.0	20.0	20.3	1.5
4.0	4.0	20.0	20.0	0.0
4.0	4.0	0.0	0.0	0.0
8.0	4.0	0.0	0.0	0.0

Discussion

Roe and Kahn (**6**) use the same principle in their colorimetric method, but work with the precipitated phosphate instead of the excess phosphate. Their paper is the only one found which was at all similar to the method of the present paper, and their results simply give further proof of the accuracy of the method used in this paper.

The main objection to the method of Roe and Kahn is that centrifuging and special technique are required in washing out the excess phosphate, which increases the labor and endangers accuracy. Incomplete washing or a slight dissolving of the phosphate is likely to cause error.

Special care must be taken to wash out excess phosphate if colloidal hydroxes of iron, manganese, and aluminum are present, since they would tend to retain the phosphate by adsorption. They will also dissolve along with the phosphate and interfere in the colorimetric phosphate test. Other interfering substances precipitated by NaOH would also be dissolved.

A large excess of phosphate does not seem necessary so long as a sufficient excess of OH^- ions is present. Of course, the OH^- ions should not be too

concentrated, since there is danger of redissolving the calcium phosphate; but this is not possible in the procedure here presented, since pH 13 or more is necessary according to ROE and KAHN, and the concentration of the NaOH here used is only slightly more than 0.1 N. No phosphate test could be secured in the filtrate when a solution containing 0.2 mg. of phosphorus was treated with 0.8 mg. of calcium and 0.1 normal NaOH and filtered. This proves that the complete precipitation of calcium phosphate does not depend on the common ion effect in the presence of OH^- ions, and only a small excess of phosphate as used in the present method would insure accurate results.

The principle of reducing color has been used previously for determining potassium (**3**) and carbon dioxide (**2**). This principle deserves as much recognition in colorimetry as titration of excess acid and alkali does in volumetric analysis. As yet its use is very limited.

The mathematical progression involved makes the colorimetric readings as the color is progressively reduced increasingly accurate. As the calcium takes out the phosphate the readings become very accurate. It is apparent that by adjusting the phosphate properly, colorimetric readings more accurate than with the ordinary increase in color intensity can be secured, since such readings approach infinity as the color loses intensity while the ordinary colorimetric readings approach zero with lessened color intensity.

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BRIEF PAPERS

POSSIBLE CHANGES IN THE WAXLIKE COATING OF APPLES CAUSED BY CERTAIN SPRAY AND OTHER TREATMENTS[1]

A number of investigators have studied the effects of arsenic, sulphur, and copper sprays on the chemical composition of fruits, particularly citrus fruits and apples. With citrus fruits (**1, 3, 4, 5, 8, 11, 12**) it has been definitely established that the use of arsenical sprays causes certain changes in acid, sugar, and vitamin C, while in the case of apples (**2**) a possible effect of certain fungicides upon the chemical composition has been indicated. Furthermore, results obtained by other workers (**6**) who have tried to determine the direct effects of the use of oil seem to indicate that its application affects the physiological behavior of the plant.

In view of the wide-spread interest and importance attached to these studies, it seemed desirable to report data dealing with possible changes in the waxlike coating of apples caused by certain spray and other treatments. While it is difficult, at the present time, definitely to evaluate the effect of changes in the condition of the cuticle on the general metabolism within the fruit, it appears very probable that skin condition may play an important rôle through its indirect effect on respiratory activity.

Besides being of interest in connection with the physiological behavior of the plant, the study of the effect of different spray treatments on the apple is of particular significance in connection with the spray-residue removal problem. In the west a large percentage of the harvested apple crop is washed with chemicals to reduce the quantity of arsenic remaining from spray materials to the amount permitted by law, and it is claimed that the successful removal of the residue is often prevented or rendered difficult by the use of oil as a component of the spray mixture used for the control of codling moth. One of the explanations offered (**10**) to account for this difficulty is based on the assumption that the oil from the spray mixture coats the lead arsenate particles and protects them from the solvent action of the acid employed to remove the arsenic. While it is known that mineral oil penetrates leaves by way of the stomata (**6**), it is not definitely known whether the oil sprayed on apples remains on the surface admixed with the natural waxlike coating or volatilizes before harvesting time, as claimed by REGAN (**9**). In one of the apple cleaning processes used, the fruit is first treated with an alkaline wash at about 110° F., then washed, brush-dried, and atomized with a slightly warmed

[1] From the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, in cooperation with the Food Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

mixture of mineral oil and paraffin wax. Examination of the cuticle of apples thus treated should indicate the effect of such treatment on the quantities of natural waxlike constituents.

Through the kindness of F. L. OVERLEY and A. SPULER, of the Washington Agricultural Experiment Station, it was possible to examine Jonathan apples from Wenatchee, Washington, some of which were unsprayed and others of which were subjected to different spray treatments. All of the trees from which these samples were collected were located rather close to one another in the same orchard. Fruit was sampled within a few hours after harvesting. Two samples of Jonathan apples grown in Yakima, Washington, were also examined, one before, and the other after, receiving a treatment consisting of an alkaline wash followed by atomizing treatment with mineral oil and paraffin wax.

The results of this study are presented in table I. The methods used in sampling and in determining the apple surface constituents, namely, ursolic acid, oily fraction, and total ether extract, are the same as those previously reported in detail (7). The method of determining cutin will be reported in a later paper. Briefly, it consisted of hot acid and alkali extraction of the cuticle, after extraction with ether and alcohol, and saponification of the remaining cutin with alcoholic potash, the loss in weight after saponification being considered the amount of cutin.

It will be seen from an examination of the data that individual as well as averaged values for ursolic acid, oily fraction, and total ether extract representing the non-oil-sprayed and the glycerin and oil-sprayed fruit are higher than corresponding values representing the check or unsprayed fruit. With but few exceptions the individual values are higher for the oil-sprayed than for the non-oil-sprayed samples. In those cases where cutin was determined the oil-sprayed samples show higher values than the non-oil-sprayed samples and the unsprayed fruit. With fruit treated with an alkaline wash and then atomized with a mixture of mineral oil and paraffin wax, the oily fraction appears to be increased by the applications.

The data indicate that the ursolic acid and oily fraction increase in approximately the same proportions; therefore it is not believed that any appreciable increase in the naturally occurring oily fraction can be ascribed to a direct accumulation on the surface of the apple of mineral oil from the spray mixture, provided the mineral oil is of relatively low viscosity and the oil spray is not applied too late in the season. It seems, therefore, that the difficulty experienced in removing spray residues by cleaning processes cannot be attributed directly to an accumulation of mineral oil from the applied spray, but rather to an increase of all the ether-soluble waxlike constituents resulting from a physiological stimulation. It should be borne in mind, however, that the application of mineral oil to the surface of the fruit might

TABLE 1

EFFECT OF SPRAY AND OTHER TREATMENTS ON THE SURFACE CONSTITUENTS OF JONATHAN APPLES

SOURCE AND TREATMENT	CONSTITUENTS PER 100,000 SQUARE MILLIMETERS OF SURFACE AREA				OILY OR PETROLEUM-ETHER SOLUBLE FRACTION IN TOTAL ETHER EXTRACT
	URSOLIC ACID	OILY OR PETROLEUM-ETHER SOLUBLE FRACTION	TOTAL ETHER EXTRACT	CUTIN	
	mg.	*mg.*	*mg.*	*mg.*	%
WENATCHEE, WASHINGTON:					
Unsprayed	481.0	321.5	802.5	946.4	40.0
Lead arsenate* and spreader,‡ calyx and covers 1–6	531.6	361.2	892.8	.....	40.5
Lead arsenate* and spreader,‡ calyx and covers 1–6	537.4	369.4	906.8	.....	40.7
Lead arsenate,* calyx and covers 2–6; lead arsenate,* pyrethrum (1–800), cover 1	567.4	334.4	901.8	969.5	37.1
Lead arsenate,* calyx and covers 1–5	516.0	339.8	855.8	888.6	39.7
Mean for non-oil-sprayed fruit	538.1	351.2	889.3	929.0	39.5
Lead arsenate,* calyx and covers 1, 2, 3, 4; oil† (70–75) and nicotine sulphate (1–1600), covers 5, 6	565.7	398.7	964.4	1080.2	41.3
Lead arsenate,* calyx and covers 1, 3, 4, 5; oil† (70–75) and nicotine sulphate (1–1600), covers 2, 6	559.2	384.2	943.4	1048.4	40.7
Lead arsenate,* calyx and covers 2, 3, 4; glycerin (1 qt.–100) and nicotine sulphate (1–800), covers 1, 5, 6	580.0	365.5	945.5	.....	38.7
Lead arsenate,* calyx and covers 1–5; lead arsenate,* oil† (70–75) and spreader (1/2–100), cover 6	573.2	360.1	933.3	1030.9	38.6
Lead arsenate* and spreader,‡ covers 1–5; lead arsenate,* oil† (70–75) and spreader, cover 6	574.2	386.2	960.7	.....	40.2
Lead arsenate,* calyx and covers 1–5; lead arsenate,* oil† (50) and spreader,‡ cover 6	561.6	398.5	960.1	.....	41.5
Lead arsenate,* oil† (50), calyx and covers 1–6	564.8	377.2	942.0	1082.6	40.0
Mean for glycerin and oil-sprayed fruit	568.4	381.5	949.9	1060.5	40.2
YAKIMA, WASHINGTON:					
Lead arsenate,* calyx and covers 1, 2; lead arsenate* and oil (1 per cent.) cover 3 about July 10	555.1	352.0	907.1	1094.7	38.8
Fruit similar to above, after alkaline wash and atomizing treatment with mineral oil and paraffin wax	554.4	410.6	965.0	1117.8	42.5

* Two pounds in 100 gallons.
† Mineral seal oil, 3/4 per cent.; figures in parentheses following oil refer to viscosity in seconds, SAYBOLT.
‡ A calcium caseinate spreader; 1/2 pound in 100 gallons.

exert a semisolvent action on the natural waxy coating, causing the lead particles to be more easily and firmly encompassed. After the disappearance of the spray oil, whether by volatilization or otherwise, the natural waxy constituents would then remain and form a more compact coating which would protect the arsenate particles against the solvent action of the cleaning fluid. Such action would be much more noticeable on varieties which are characterized by unusually high quantities of natural waxy coating, such as Arkansas Black and Esopus Spitzenburg.—K. S. MARKLEY AND CHARLES E. SANDO, *U. S. Department of Agriculture, Washington, D. C.*

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GAS INJURY TO PURE CULTURES OF *SPIRODELA*

An instance of severe injury to *Spirodela polyrhiza* (duckweed) growing in pure culture in a modified Knop's solution is here reported. This injury was caused by introducing small amounts of illuminating gas into the culture flask during transfer of the plants from one flask to another. The cultures were maintained in a sterile dilute Knop's solution, 250 cc. per 500-cc. flask, and were protected by a cotton plug over which was placed an inverted tall beaker (150 cc.). The latter was used to protect the stopper from dust, as it is advisable to have loose plugs for this type of work, on account of possible interference with gas exchange.

During the transfer of a single plant from one culture flask to another there was severe injury, not only to the transferred plant, but also to the culture from which the transfer had been made. The latter was sometimes lost entirely. The cotton was always flamed before removal, and again after it had been replaced. This was thought necessary because the cultures are often maintained for many weeks and are exposed to dust during that time. The interior of the beaker was flamed while held in an inverted position and was quickly placed over the cotton plug. The open neck of the flask was never flamed directly. Following this procedure the plants were almost always severely injured, as shown by abscission of the roots, separation of the plants into their component units, and death of nearly all the green portions except the region around the growing point at the node. After from one to two weeks the injured plants regenerated new leaves and roots, again producing normal colonies. Under these circumstances the intended experiments could not be made.

After some attempts to determine the cause of the injury, it was finally learned that unburned or partly burned gas was being introduced into the flask when the flamed inverted beaker was replaced over the neck of the flask. When flaming was omitted no injury whatever occurred, not even the loss of roots, which, in *Spirodela,* is a most sensitive indicator of injury.

Since flaming of the neck of sterile culture tubes and vessels is a procedure that has been much followed, although discouraged by many technicians, it is possible that occasional failure of growth in cultures is sometimes the result of this precautionary technique. Such injury would be more evident in those instances in which small numbers of individuals are transferred. It is possible that gas injury may have been encountered by others during the transfer of pure cultures of green plants or of bits of fungus mycelium.

Since *Spirodela* is sensitive to injury by fuel gas, an experiment was made to determine the relation of time of exposure to degree of injury. Pure cultures of vigorously growing plants were exposed to an atmosphere

consisting entirely of illuminating gas. This was a carburetted water gas with an illuminant content of 10.2 per cent. After exposure the gas was removed by a stream of air passed into the flask until no more odor was detected. Exposures were made for 5, 7, 8, and 10 minutes. Within less than an hour later severe injury could be detected in all cases. The leaves became a pale whitish green, indicating chlorophyll decomposition, the surface became slightly concave (in contrast with the normal slightly convex appearance), and numerous drops of liquid were exuded on the upper epidermis, most numerous about one-third the distance from the node to the tip. Later the roots separated from the plants. All plants after the 10-minute exposure were killed, and but few survived after a 5-minute exposure.

It is suggested that *Spirodela* may be used in a technique for detecting the presence of illuminating gas in soils, where high concentrations are often present as the result of leaks in gas mains. TRUE[1] has described a technique for this purpose, using the sweet pea. It is doubtful whether *Spirodela* is as sensitive to traces of illuminating gas or ethylene as those plants studied by CROCKER[2] and his coworkers.—ALBERT SAEGER, *Cornell University, Ithaca, New York*

OCCURRENCE OF DULCITOL IN *IRIDEAE LAMINARIOIDES* (RHODOPHYCEAE)

In studying the nature of the cell wall constituents of *Irideae laminarioides,* which grows abundantly on the rocks of the Pacific Coast, at Moss Beach, California, the investigation was primarily concerned with a polysaccharide, galactan, which consists chiefly of galactose units. The writer was also interested in determining whether or not the metabolism of this plant is based on a sugar such as glucose or sucrose, as in the higher plants, or on a carbohydrate other than these.

Material was collected at four different times of the year, on May 12, June 20, August 28, and October 27 of 1932. The plants were extracted with alcohol and analyzed for reducing and total sugars. All of the samples failed to give a reducing value with Fehling's solution either before or after hydrolysis. On concentrating the alcoholic extract, however, a thick syrup was obtained.

Since the presence of mannitol in brown algae was reported by KYLIN (**5, 6**) in 1913–1915, and more recently by HAAS and HILL (**4**), it seemed likely that this syrup might contain the alcohol. Its isolation was therefore

[1] TRUE, R. H. Florists Exchange **73**: 13. 1930.

[2] CROCKER, WM. Florists Exchange **70**: 15. 1929. Also in Boyce Thompson Inst. Prof. Papers **11**: 81–85. 1929. And later publications in Contrib. Boyce Thompson Inst.

attempted. The plants were boiled for 15 minutes in 95 per cent. alcohol immediately after collection, then dried in a vacuum oven at 40° C. One hundred and fifty grams of the dry tissue were ground up and sieved. The ground material was then extracted in a large Soxhlet with 80 per cent. alcohol, and this extract was combined with the alcoholic portion in which the plants had been boiled immediately after collection. The combined solution was treated with lead acetate, the excess of lead removed with 2 per cent. H_2SO_4, and the lead sulphate filtered off. The filtered solution was then treated according to the method of HAAS and HILL (**4**) for preparation of mannitol, but no crystalline form of this alcohol could be obtained from the syrup. A portion of the syrup was placed in the vacuum oven and concentrated at 80° C. for 12 hours, the syrup then being weighed and its specific rotation observed. The substance showed no rotatory power; also, the addition of borax (**1**) to this solution did not increase its rotation. This fact furnished additional proof of the absence of mannitol.

Since the *Irideae laminarioides* contained a considerable amount of galactan, it was suspected that the sugar alcohol of galactan, dulcitol, might be present. The syrup was extracted again in a Soxhlet with absolute alcohol, the extract clarified with charcoal, concentrated to a syrup, and allowed to stand for about a week with occasional stirring. White crystals began to separate. After recrystallization of these crystals and examination, the following was observed: the specific rotation of this substance, $[\alpha]_D = 0$; melting point 185°; oxidation with nitric acid yielded mucic acid. These facts constitute conclusive proof that this substance isolated from *Irideae laminarioides* was dulcitol.

HAAS and HILL (**2, 3**) have recently reported the occurrence of dulcitol and sorbitol in a red alga, *Brostrychia scorpioides*. On examination of eight other species of Rhodophyceae, however, they found no evidence of the presence of either dulcitol or sorbitol.

The isolation of dulcitol from *Irideae laminarioides*, therefore, confirms the occurrence of dulcitol in red algae found by HAAS and HILL.

The polysaccharide galactan isolated from *Irideae laminarioides* is precipitated out from 95 per cent. alcohol and is obtained in the form of threads. It absorbs many times its own weight of water and forms a colloidal solution. It gives no reducing value, but after hydrolysis with 2 per cent. sulphuric acid it strongly reduces Fehling's solution. Upon oxidation with nitric acid it yields mucic acid. This polysaccharide is being investigated at the present time.

It is conceivable that a possible equilibrium exists between the polysaccharide, galactan, and the sugar alcohol, dulcitol. This may be analogous in the carbohydrate metabolism of the plant to the equilibrium that exists

between starch and glucose in higher plants. This suggestion supports the idea that the metabolic process of some of the algae may depend upon carbohydrates other than glucose or sucrose. In the case of *Irideae laminarioides* this is probably dulcitol.—W. Z. HASSID, *University of California, Berkeley, Calif.*

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1. BROWNE, C. A. Handbook of sugar analysis. Pp. 766–767. John Wiley & Sons, New York. 1912.
2. HAAS, P., and HILL, T. G. The occurrence of sugar alcohols in marine algae. Dulcitol. Biochem. Jour. **25**: 1470–1741. 1931.
3. ————, and ————. Biochem. Jour. **26**: 487–000. 1932.
4. ————, and ————. An examination of the metabolic products of certain fucoids. II. Mannitol and mannitan. Biochem. Jour. **23**: 1005–1009. 1929.
5. KYLIN, H. Zur Biochemie der Meeresalgen. Zeitschr. physiol. Chem. **83**: 171–197. 1913.
6. ————. Untersuchungen über die Biochemie der Meeresalgen. Zeitschr. physiol. Chem. **94**: 337–425. 1915.

MOVEMENT OF ORGANIC MATERIALS IN PLANTS: A CORRECTION

In a recent note upon the mechanism of translocation,[1] the writers criticized the use made by CRAFTS[2] of the Poiseuille expression for uniform, non-turbulent, viscous flow in a capillary of known dimensions. To this criticism in all its general aspects we still adhere, especially in so far as it refers to the inapplicability of the formula in question to a "flow" which is clearly not uniform, and also to the comparison made between the whole phloem wall substance and the pores in the sieve plates as possible avenues for translocation. The basis of the latter criticism is that CRAFTS by treating the whole phloem wall substance as a single, circular capillary, derived pressures which can have no possible relation to the actual pressures involved in the production of a flow of the desired dimensions in the phloem wall. These are unjustifiably compared with pressures calculated to refer to flow through the pores in the sieve plates.

In the attempt to pursue CRAFTS' own method and insert a dimension (one half the mean wall thickness) which, on the assumption of flow

[1] STEWARD, F. C., and PRIESTLEY, J. H. Movement of organic materials in plants: A note on a recently suggested mechanism. Plant Physiol. 7: 165–171. 1932.

[2] CRAFTS, A. S. Movement of organic materials in plants. Plant Physiol. 6: 1–38. 1931.

through the whole wall, would lead to a more probable pressure an error has been made, an error which we have realized following correspondence with Dr. CRAFTS and which we now desire to correct. The treatment (p. 167) which actually led to a factor $\frac{r}{(r_1)^4}$ by which CRAFTS' derived pressures should be multiplied ought to have led to the factor $\frac{r}{(r_1)^2}$. It will be clear from the context, however, and from the subsequent discussion (which points out that even thus modified the treatment cannot be accepted), that this error does not materially affect our general criticisms.—F. C. STEWARD and J. H. PRIESTLEY, *University of Leeds, England.*

NOTES

Annual Election.—The results of the annual election of the American Society of Plant Physiologists have just been announced by the Secretary-Treasurer as follows: President for 1933–1934, Dr. C. O. Appleman, University of Maryland; Vice-President, Dr. H. R. Kraybill, Purdue University; Secretary-Treasurer for 1933–1935, Dr. A. E. Murneek, University of Missouri. The new officers inherit some difficult problems arising from the depression. In attempting to solve these problems, they will need and expect cooperation from the entire membership. The same optimism and courage that made possible the establishment of a good journal for publication of research will be able to conquer the present difficulties.

Summer Meeting.—The summer meeting of 1933 was held in Chicago on June 20–22, in connection with the programs of Sections G and O, A. A. A. S., the Botanical Society of America, the American Phytopathological Society, and the American Society of Agronomy. The special meeting for plant physiologists was a symposium on "Radiation and Plant Life" on June 22. Lively discussion of the papers featured the meeting, and enhanced the value of the conference.

Boston Meeting.—The tenth annual meeting of the Society will be held in Boston in December, 1933. The program will be arranged by a program committee, as usual. The chairman of this committee is Dr. B. E. Gilbert, Director of the Rhode Island Agricultural Experiment Station. Prompt response to requests of the committee will be greatly appreciated.

Membership Committee.—A large membership committee was appointed by President D. R. Hoagland, with Dr. Laurenz Greene, of Purdue University, as chairman. The October number of Plant Physiology will be small, since it is necessary to utilize reserves in order to provide an October issue. Those who drop subscriptions during the depression are voting to discontinue the facilities for publication of research. At the same time, authors are more urgent about the appearance of their papers than in normal times. With more material on hand than can be used promptly, each member of the society should aid the membership committee to enlarge our funds for publication. Every new member helps just that much. Each fee pays for one page of printed matter. A few orders for back volumes would be valuable in providing larger funds for immediate use. It is not a question of whether one can afford to subscribe for journals at this time, but whether we can afford not to maintain the usual outlets for

publication of material. Research is without much value until it has been published and its results utilized by mankind.

Purdue Section.—The Purdue Section of the American Society of Plant Physiologists has just completed another successful year. There are 32 members, 11 of whom are members of the national organization. The average attendance has been about 25 at each biweekly meeting. Aside from the regular program, two dinner meetings were held. The first was a joint meeting with the Purdue Section of the American Chemical Society at which Dr. K. P. LINK, of the University of Wisconsin, gave an interesting discussion on the biochemistry of certain plant diseases. The speaker at the final meeting was Dr. I. L. BALDWIN, of the University of Wisconsin, who discussed the legume or root nodule bacteria.

The program for the regular meetings was as follows:

November 7, Chemical and varietal studies of the consistency of canned pumpkin. D. M. DOTY.

November 21, Separation and identification of viruses occurring in solanaceous plants. R. W. SAMSON.

December 5, Virus diseases of various species of *Prunus*. J. A. MCCLINTOCK.

January 16, Joint evening meeting with the Biological Society. Reports of the Atlantic City meeting of A. A. A. S.

February 6, Some effects of light on the growth of asters. M. W. RICHMAN.

March 6, Recent studies on cold tolerance of corn. J. R. HOLBERT, U. S. Department of Agriculture.

March 20, Root development in hardy and non-hardy wheat varieties. W. W. WORZELLA.

April 17, Effects of various salt solutions on respiration in seeds. W. W. JONES.

At the last regular meeting the following officers were elected for the year 1933–1934: Dr. G. N. HOFFER, Chairman; Mr. R. B. WITHROW, Secretary and Treasurer.—W. W. JONES, Secretary.

International Critical Tables.—An index has finally been provided for this important work. The price quoted to subscribers is $3.00 for this volume. The McGraw-Hill Book Co. has rendered a great service to science in the publication of these tables, which are now rendered much more useful through careful indexing of the entire work in one volume.

Handbook of Plant Analysis.—The third volume of G. KLEIN's Handbuch der Pflanzenanalyse was published by the Vienna plant of Julius

Springer. It is an immense volume, 1613 pages, bound in two separate parts. The first half, 806 pages, contains methods of analysis for membrane substances, natural tannins, lichen acids, ethereal oils, caoutchouc and gutta-percha, and resins. The second half, 807 pages, is devoted to many types of glucosides (aliphatic and aromatics, flavones, anthocyanins, anthracenes, hydrocyanic, indoxyl, mustard oils, saponins, digitalis, and other obscure types), and pigments (carotinoids, chlorophyll, algal pigments, fungal and bacterial pigments, and other slightly studied pigments). An appendix treats lignin analysis. The index alone occupies 137 pages. It is not possible to describe the work in detail here. It is an extremely compendious handbook, and represents an enormous amount of labor on the part of the authors, editor and publishers. Orders may be sent to J. Springer, Schottengasse 4, Vienna I. The price of this volume in two parts is RM 162 unbound, RM 168 bound in cloth.

Manual of Plant Biochemistry.—Plant physiologists and biochemists will be interested to know that Dr. W. E. TOTTINGHAM is preparing a manual of plant biochemistry which will be published in a few months by the Burgess Publishing Co., 519–521 Second Ave. So., Minneapolis, Minnesota. Only a portion of the work has been examined, and no price can yet be quoted. Inquiries can be addressed to Mr. C. S. HUTCHINSON, of the Burgess Co.

Microbiology of Forest Soils.—The microflora and microfauna of forest soils are unique. A volume of D. FEHÉR, Untersuchungen über die Mikrobiologie des Waldbodens, has been published by J. Springer, Berlin. It contains eleven chapters, 272 pages. There is a table of contents, but no index. Methods of research are described, bacteria of forest soils, production of CO_2, nitrogen cycle, seasonal changes in biological transformations, microbiological characteristics of sandy forest soils, fungi and algae in European forest soils, and protozoa of forest soils. An appendix deals with the special microbiological problems of alkali soils. The chapter on protozoa is by L. VARGA, and the alkali (Szik-Boden) soils are discussed by R. BOKOR. The work is a very valuable contribution, represents ten years of research in the Institute of which FEHÉR is the director, and brings much new information into brief compass. The price quoted is RM 24 for paper binding. Orders should be addressed to J. Springer, Linkstrasse 23–24, Berlin W9.

Principles of Plant Physiology.—A revised edition of this work by Dr. O. L. RABER has been published by Macmillan Co., at $3.00 per copy. The general plan of the work remains unchanged. On the assumption that

we know more about aerobic respiration than anaerobic, the former is treated first, as a "known" from which to approach the "unknown." The facts of anaerobic respiration, however, are probably better substantiated than those of aerobic respiration, if we consider respiration as a chemical process. Students will find it a helpful introduction to plant physiology. Citations of recent literature have been liberally used. The book includes portraits of a number of living plant physiologists and plant chemists. Some errors that marred the first edition have been eliminated.

Plant World in Florida.—A very attractive book, the collection of the notes and writings of Dr. HENRY NEHRLING, who died in 1929 after a long experience with Florida's flora, has been edited by ALFRED and ELIZABETH KAY. It carries a preface by DAVID FAIRCHILD, whose explorations were so delightfully presented to lovers of nature a couple years ago. Many groups of plants are described, always in alphabetic order within the group, so that it is easy to find the description and notes concerning any important member of the Florida vegetation. A fine portrait of Dr. NEHRLING adorns the work as a frontispiece. The book was sponsored by the Garden Club of Palm Beach. The price is $3.50 per copy, and orders for it can be sent to the publishers, Macmillan Co., New York.

Plant Names.—The Macmillan Co. has also published a little volume by Dr. LIBERTY HYDE BAILEY, on How Plants Get Their Names. Many botanists will be helped by this work, especially beginners who wish to understand the problems of nomenclature. It is written in very simple language, so that even a layman can grasp it. A long list of specific names with their pronunciations, and with their probable meanings in plain English, is a valuable feature of this handy little volume. It is quoted by the publishers at $2.25 per copy.